VALIDITY OF BODY COMPOSITION ASSESSMENT IN RACIAL AND ETHNIC MINORITY POPULATIONS

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ABSTRACT

Malia Nikole Melvin Blue: Validity of Body Composition Assessment in Racial and Ethnic Minority Populations
(Under the direction of Abbie E. Smith-Ryan)

Few investigations have evaluated the validity of up-to-date body composition technology across diverse populations. Due to the relationship between obesity and cardiometabolic disease risk, it is vital to measure body composition accurately. The overall purpose of the proposed study was to assess the validity of multiple body composition assessments utilizing a four-compartment model criterion within a multi-ethnic sample stratified by race/ethnicity. One hundred and ten individuals (55% female, Age: 26.5 ± 6.9, body fat percentage [%fat]: 25.7 ± 9.5%) identifying as Asian (n=22), African American/Black (n=22), Caucasian/White (n=22), Hispanic (n=22), Multi-racial (n=21) and Native American (n=1) were enrolled in the present study. Eight body composition models were evaluated including dual energy X-ray absorptiometry (DXA), air displacement plethysmography (ADP), two bioelectrical impedance devices (BIS, IB) and four multi-compartment models (BIS 4C, BIS 3C, deuterium dilution 3C, DXA-body volume [BV] 4C) which utilized a combination of the single device estimates to measure body composition (%fat, fat-free mass [FFM]). For the total multi-ethnic sample, measures of %fat and FFM from multi-compartment models were all excellent to ideal (%fat: TE=0.94–2.37%; FFM: TE=0.72–1.78 kg), with the exception of the DXA-BV 4C model, which was good to fairly good for %fat (TE=3.79%). For the single device models, %fat measures were very good to excellent for DXA, ADP and IB (TE=2.52-2.89%) and fairly good for BIS (TE=4.12%). For FFM, single device estimates were very good to ideal. Results did not

vary significantly between races/ethnicities. The current study results suggests the multi-compartment models evaluated can be utilized in a multi-ethnic sample, as well as in each individual race/ethnicity to obtain highly valid results for both %fat and FFM. Additionally, the single device estimates from DXA, ADP and IB are valid for mean estimates. The BIS may not be valid for estimates in African American/Black, Caucasian/White and Multi-racial samples. Investigators and clinicians can accurately estimate body composition in minority populations utilizing the devices evaluated in the present study, however, BIS results should be interpreted cautiously.

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LIST OF ABBREVIATIONS

%fat	Body fat percentage
2C	Two-compartment
3C	Three-compartment
4C	Four-compartment
ADP	Air displacement plethysmography
BIS	Bioelectrical impedance spectroscopy
BM	Body mass
BMC	Bone mineral content
BMI	Body mass index
B-mode	Brightness mode
BV	Body volume
D2O	Deuterium oxide used for Deuterium dilution
DXA	Dual energy X-ray absorptiometry
ECF	Extracellular fluid
EI	Echo intensity
FFM	Fat free mass
FL	Muscle fascicle length
FM	Fat mass
IB	Inbody 770 bioelectrical impedance device
ICF	Intracellular fluid
mCSA	Muscle cross sectional area

Mo	Bone mineral
MT	Muscle thickness
MV	Muscle volume
PA	Muscle pennation angle
TBW	Total body water
US	Ultrasound
VAT	Visceral adipose tissue
VL	Vastus lateralis

CHAPTER I: INTRODUCTION

1.1 Body Composition Assessments

The high prevalence of overweight and obesity is a persistent public health concern in the United States [93]. Overweight and obesity has been shown to increase the risk for developing several chronic conditions including metabolic syndrome, cardiovascular disease, diabetes, and cancer [17,43,99]. Body mass index (BMI) is widely used in clinical settings to assess obesity and the associated health risks. However, BMI is incapable of differentiating between the various components of body composition and may mischaracterizes overweight and obesity. Body composition models were established to quantify the different compartments of body mass. Two-compartment (2C) models such as air displacement plethysmography, bioelectrical impedance analysis/spectroscopy, skinfold measurement, and hydrostatic weighing divide the body into fat mass (FM) and fat free mass (FFM), and are the most common forms of body composition assessment. However, the validity of each 2C method is dependent upon several factors related to the sample population, pre-assessment guidelines, and meeting required assumptions (i.e. fat free body density, body proportions, hydration, etc.)[36]. Multi-compartment models are considered the criterion method for analysis of body composition [50,149], as they measure additional constituents of the body such as total body water, bone mineral content, and soft tissue mineral content. A comprehensive study by Wang et al. (1998) investigated the validity of 16 body composition models compared to the 6-compartment neutron activation model and found multi-compartment models that include total body water estimates demonstrated the lowest total error and highest reliability. Total body water is the largest molecular component which emphasizes the importance of accurate total body water estimates.

1.2 Health and Body Composition in Minority Populations

Minority populations in the United States are at an increased risk for numerous chronic diseases including obesity and related comorbidities including hypertension, cardiovascular disease and metabolic syndrome [63,95,133]. Investigations have observed racial and ethnic differences in body composition and muscle physiology, potentially contributing to elevated disease risk. The relationship of fat distribution, particularly visceral fat (VAT) [164] and intramuscular fat [121], to cardiometabolic health is important to consider in minority populations. Previous data reported differences between black, white and Hispanic adults in trunk and segmental fat and lean mass (LM) distribution [53]. Additionally, recent investigations evaluating VAT in minorities demonstrated significantly greater VAT in white males compared to black males [52], and significantly greater VAT in South Asian individuals in comparison to Chinese Americans, African American and Latinos [120]. South Asian participants were also found to have significantly greater intramuscular fat in comparison to all other racial groups studied.

Understanding the relationship between body composition phenotypes and health in minority populations is vital. However, the racial/ethnic variations in body compartments may influence the ability of body composition models, especially 2C models, to accurately assess body composition. In anthropological studies, Black individuals have been found to have longer extremities and a shorter trunk region [139]; limb length is particularly important for accurate estimations of total body water and FFM from bioelectrical impedance spectroscopy (BIS). Several studies have performed regression analyses to establish an accurate prediction of body composition estimates using bioelectrical impedance analysis in non-Caucasian populations [15,28,45]. However, currently, there is no consensus on the most appropriate method for accurately estimating body composition with bioelectrical impedance devices in multiple races. Recent studies that have investigated the validity of body composition models in racial/ethnic minorities are limited. Few studies evaluating minority populations have used a criterion multi-compartment model to determine validity of body composition techniques [15,28,45]. Furthermore, the majority of previous studies have included only one racial/ethnic category [131,137,145,156], and assessed the validity of a single body composition technique.

1.3 Statement of Purpose

The primary purpose of the proposed study was to assess the validity of multiple body composition assessments utilizing a multi-compartment criterion within a multi-ethnic sample stratified by race/ethnicity.

1.4 Specific Aims

Specific Aim 1: To assess the validity of existing body composition models compared to a 4-compartment (4C) criterion model for measures of body fat percentage (%fat), fat free mass (FFM) and fat mass (FM) in a multi-ethnic sample stratified by race and ethnicity.

- The 4C criterion model utilized air displacement plethysmography (ADP) to assess body volume (BV), deuterium dilution (D_2O) to measure total body water (TBW), and dual energy X-ray absorptiometry (DXA) to measure bone mineral content.
- The validity of two 4C models were compared to the traditional 4C criterion: Model 1: TBW was measured by bioelectrical impedance spectroscopy (BIS); Model 2: BV was estimated by DXA-derived coefficients and TBW measured by BIS.
- The validity of two 3C models utilizing BV and TBW estimates were evaluated: Model 3: BV was assessed by ADP and TBW was measured by D_2O; Model 4: BV was assessed by ADP and TBW measured by BIS.
- Three 2C models and one single-device 3C model were assessed for validity compared to the criterion: ADP, BIS (SFB7 ImpediMed), BIS_2 (InBody 770) and DXA.

Specific Aim 2: To assess the validity of two multi-frequency bioelectrical impedance devices for measures of total body water compared to deuterium dilution in individuals stratified by race and ethnicity.

- Deuterium dilution estimates of TBW was the criterion. Two bioelectrical impedance devices ($BIS_{1,}$ $BIS_{2,}$) estimates of TBW were assessed for validity compared to the criterion.

Specific Aim 3: To assess the validity of bioelectrical impedance devices compared to DXA for measures of segmental FFM in a multi-ethnic sample and stratified by race and ethnicity.

- The validity of segmental (arm and leg) FFM values from BIS devices was compared to DXA measures of segmental FFM as the criterion.

Exploratory Aim: To characterize and compare body composition and muscle characteristics between each racial/ethnic cohort.

- Body composition (FM, FFM, %fat, bone mineral density [BMD], visceral fat [VAT], android/gynoid distribution) and muscle characteristics (muscle volume, size, quality and architecture) were assessed for all racial/ethnic cohorts.

1.5 Research Questions

- Research Question 1: Are existing body composition models valid for the assessment of body composition (%fat, fat mass, fat free mass) in a multi-ethnic sample and within each race/ethnicity?
- Research Question 2: Is there a difference in the validity of existing body composition models between individuals of different races/ethnicities?

- Research Question 3: Are bioelectrical impedance devices valid for the assessment of total body water in a multi-ethnic sample stratified by race and ethnicity?
- Research Question 4: Are bioelectrical impedance devices valid for the assessment of segmental body composition in a multi-ethnic sample stratified by race and ethnicity?
- Research Question 5: Is there a difference in body composition characteristics between races/ethnicities?

1.6 Research Hypotheses

Hypothesis 1A: Multi-compartment body composition models will be valid (Total error [TE] < 2.5 kg FFM) for the multi-ethnic sample and within each race/ethnicity.

Hypothesis 1B: Multi-compartment models that include total body water will have better accuracy (TE < 1.8 kg FFM) than models that do not assess total body water.

Hypothesis 2A: Validity of 2C body composition models will be less accurate (TE > 3.5 kg FFM) in African American, Asian and Multi-racial individuals.

Hypothesis 2B: There will be no difference in the validity of 2C body composition models between Hispanic, Caucasian and Native Americans.

Hypothesis 3A: Bioelectrical impedance assessments will not be valid (TE >2 L) for the estimation of total body water in African Americans and Multi-racial participants.

Hypothesis 3B: Bioelectrical impedance assessments will accurately (TE < 2 L) predict total body water in Caucasian, Asian, Hispanic and Native American participants.

Hypothesis 4A: Bioelectrical impedance assessments that include segment length will be valid for the estimation of segmental FFM (TE < 0.8 kg) in all participants.

Hypothesis 4B: Bioelectrical impedance assessments that do not include segment length will not be valid (TE > 1.5 kg) for the estimation of segmental FFM for African Americans, Hispanics, Asians or Multi-racial participants.

Hypothesis 5: Body composition and muscle characteristics will significantly differ between racial/ethnic cohorts.

1.7 Assumptions

Theoretical

- All participants arrived 12 hours fasted and euhydrated and had abstained from alcohol, exercise and caffeine.
- All participants accurately reported self-identified race/ethnicity.
- Participants in the Asian, Black/African American, Hispanic and White/Caucasian were >50% of the self-reported race
- Participants enrolled were representative of each racial category in the U.S.

- The hydration constant of 0.732 was appropriate for all participants; 73.2% of FFM is composed of water.
- For estimates of TBW by bioelectrical impedance spectroscopy, it was assumed participants have a standard body proportion (coefficient = 4.30) and body density (1.05 kg/L).
- The propagation of errors of a multi-compartment model did exceed the accuracy achieved by assessing multiple body constituents.

Statistical

- The population from which the sample is drawn was normally distributed.
- Sample variability was equal.

1.8 Limitations

- Results may not be generalizable to all individuals within a racial/ethnic cohort, specifically children and elderly.
- Results may not generalizable to highly trained individuals (elite athletes) or individuals with chronic conditions that influence body composition components (renal failure, muscle wasting, osteoporosis)
- The study sample was powered to assess validity in a multi-ethnic sample, but was not fully powered to assess differences between races/ethnicities.

- Genetic and cultural differences between races/ethnicities that may influence body composition phenotype and validity of measures were be assessed.

1.9 Significance of Study

The high rates of obesity, cardiovascular and metabolic disease in minority populations requires a reevaluation of our ability to assess and manage body composition effectively. According to the U.S. Census Bureau, minority populations, including individuals who identify with two or more races, are increasing. Within the next 40 years, over 50% of the U.S. population will consist of individuals who identify as a racial/ethnic minority. However, minorities are underrepresented in biomedical research with as low as 2-27% representation in various fields [94,111]. Oftentimes, body composition studies do not report the racial composition of the participants evaluated. The omission of race/ethnicity is important to consider as compartments of the body may vary slightly depending on race and ethnicity [139]. Differences in body constituents may influence the accuracy of body composition estimates. Therefore, individuals of understudied races/ethnicities may be improperly categorized for health risk due to the poor validity of body composition methods utilized. The aim of the proposed study, to investigate the validity of several existing body composition techniques, will allow for better assessment and translation to minority populations. Results will help inform researchers and practitioners of the most appropriate method for body composition estimation considering race, ethnicity, as well as feasibility. Improving body composition assessment is a vital initial step toward understanding the health and obesity-related disease risk for minority populations.

1.10 Operational Definitions

Air displacement plethysmography: a device utilizing a dual-chamber, sealed compartment which quantifies air displaced to measure body volume and body density.

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam [2].

Bioelectrical impedance analysis: a single or multi-frequency device that conducts an electrical current through the body and uses regression analyses to estimate total body water and body composition.

Bioelectrical impedance spectroscopy: a multi-frequency device that conducts an electrical current through the body and uses a cole-cole plot to measure extracellular fluid and total body water to estimate body composition.

Black/African American: A person having origins in any of the Black racial groups of Africa [2].

Body density: Total body mass expressed relative to total body volume [49].

Body fat percentage: Fat mass expressed as a percentage of total body weight [49].

Bone mineral content: bone mineral ash measured by dual-energy X-ray absorptiometry; BMC × 1.0436 estimates total body bone mineral [101].

Bone mineral density: the amount of bone mineral content divided by the area of bone (g/cm^2).

Body volume: Measure of body size estimated by water or air displacement [49].

Dual energy X-ray absorptiometry: a three-component model utilizing a low dose X-ray to measure total body and segmental bone mineral content, fat mass and lean soft tissue mass.

Echo Intensity: a non-invasive grayscale analysis of ultrasound measures as expressed in values between 0 and 255 a.u. to estimate the amount of intramuscular adipose and connective tissue [37].

Ethnicity: an individual's identification to a group of common ancestry, language and nation of origin.

Fat free body density: Overall density of the fat-free body calculated from the proportions and respective densities of the water, mineral, and protein components of the body [49].

Fat free mass: All residual lipid-free chemicals and tissues including water, muscle, bone, connective tissue, and internal organs [49].

Fat mass: All extractable lipids from adipose and other tissues in the body [49].

Hispanic: as a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin regardless of race [54].

Lean soft tissue: non-bone fat free mass, which includes fat free tissues such as water, muscle, connective tissue, and internal organs [89].

Limits of agreement: the 95% likely reference range for the difference between method estimations[50].

Multi-compartment model: body composition models that divide the body into three or more components such as total body water, bone mineral content, lean soft tissue [49].

Multi-racial: A person who identifies with two races, having one parent of a race and the other parent of another race.

Muscle architecture: geometric characteristics of a muscle fiber including fascicle length and pennation angle [40] that influence contractile properties of the muscle.

Muscle quality: a measure of the amount of contractile versus non-contractile tissue within the muscle [37].

Muscle size: a measure of muscle cross sectional area or muscle volume measured via magnetic resonance imaging, computed tomography or ultrasonography [92].

Native American: A person having origins in any of the original peoples of North and South America (including Central America) and who maintains tribal affiliation or community attachment [2].

Race: an individual's self-identified social group determined by racial and national origin or sociocultural group [2].

Standard error of the estimate: the degree of deviation of the individual data points around the line of best fit[50].

Total body water: A measure of the intracellular and extracellular fluid compartments of the body [49].

Total error: the average deviation of individual scores from the line of identity[50].

Ultrasound: a technique used to measure body composition by using a transducer probe to emit, through the skin, an ultrasonic wave, which part is reflected at the fat muscle interface [98].

Visceral adipose tissue: adipose tissue surrounding the intra-abdominal organs [122].

White/Caucasian: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa [2].

CHAPTER II: REVIEW OF LITERATURE

2.1 Introduction Part 1: Body Composition

Body composition assessments were established to measure the various components of body mass such as fat tissue, lean soft tissue, bone mineral content and total body water. Multi-compartment models have the ability to measure multiple constituents of the body and are considered the criterion for body composition estimation [50,148]. However, multi-compartment models require a minimum of two devices to measure additional compartments of the body. Therefore, single device two-compartment models (2C; air displacement plethysmography, bioelectrical impedance, hydrostatic weighing) and three-compartment models (3C; dual-energy X-ray absorptiometry) are commonly utilized to estimate fat mass (FM) and fat free mass (FFM). By measuring fewer body compartments, several assumptions must be met for accurate measure of body composition. Depending on the device, the validity of measures may be influenced by hydration, fat distribution, body proportions for a given height, and fat free body density [36,50]. Investigations evaluating body composition in racial and ethnic minorities have observed slight differences in fat distribution (i.e. visceral vs. subcutaneous, intramuscular fat, trunk vs. limbs) [52,53,120], fat free body density [139], and body proportions [139] between cohorts. Consequently, for racial/ethnic minority populations, estimates of body composition, especially by 2C models may not be valid. Previous body composition validation studies are inconsistent with the inclusion of minority populations and reporting of race/ethnicity. Therefore, the purpose of this review was to comprehensively examine the validity of common body composition models in African American, Hispanic, Asian, Native American and multi-ethnic individuals.

2.2 Validity of DXA

Validity within Multi-Ethnic Samples

Dual energy X-ray absorptiometry has been established as a valid method for measuring body composition, however, few studies have validated DXA within minority populations. In a multi-ethnic sample of 23 individuals (12 white, 3 black and 8 Puerto Rican), the DXA (Lunar DPX model) measures of FM demonstrated a mean difference (MD) of 1.51 ± 1.1 kg, total error (TE) of 1.31 kg, standard error of the estimate (SEE) of 1.73 kg, and coefficient of determination (r^2) of 0.972 [148]. A follow up investigation in 14 Caucasian, 5 African American and 8 Puerto Rican participants found no significant difference in %fat estimates (MD: 0.54 ± 2.4 %, r= 0.983) between DXA and the 5C model [146]. A study evaluating a fan bean DXA (Hologic, QDR 4500A) compared to a 4C model included 6 African Americans in a sample of 58 participants ages 70-79 yrs and reported a SEE = 1.6 kg for measures of FFM and a strong correlation (r=0.99) [136]. In a smaller sample, 13 participants, including 2 African Americans, reported a significant difference between the 4C body composition estimate of FFM (51.1 ± 12.4 kg) compared to the fan beam (53.7 ± 12.9 kg) and pencil beam (48.4 ± 12.1 kg) DXA estimates [136]. In a college-age sample of 62 black and 110 white males and females there was no significant differences between DXA %fat and the 4C model (MD: 0.4 ± 2.9%, r =0.94, SEE = 2.8%). In the multi-ethnic samples presented, DXA estimates demonstrated very good to excellent validity [50] compared to multi-compartment models. However, the representation of racial/ethnic minorities primarily only included African Americans and accounted for between 10 – 47% of the sample.

Validity by Race/Ethnicity

An investigation evaluating Native American females ages 18-60 yrs, reported that DXA measures of %fat demonstrated less than ideal validity compared to a 3C density model (r^2= 0.785, SEE = 3.28%, TE=3.27%) [51]. Conversely, in a study of 30 black males (19-45 yrs, BMI = 18.9-40.5 kg/m^2), DXA and 4C %fat were not significantly different (MD = -0.28%), with SEE =2.26% and r = 0.95 [140]. Additionally, DXA %fat was not significantly different from a 4C estimate (MD = 0.2 %) in 39 black males [22]. In a sample of 291 Asian (Chinese n=108, Malays n=76, Indian n=107) males and females ages 18-75 yrs and BMI between 16-40 kg/m^2, DXA estimates of %fat were underestimated compared to the 4C. Mean differences ranged between 2.1 - 2.5 % for females and between 3.2 - 4.2 % for males of the three ethnic cohorts, with a moderate correlation (F: r = 0.62; M: r = 0.56). To our knowledge, studies investigating the validity of DXA in Hispanic populations [105] and in larger multi-ethnic samples [155] have primarily been evaluated in children ages 9-17 yrs. Future investigations should evaluate the validity of DXA, particularly in adult Hispanic/Latino populations, as well as in cohorts including both sexes for black and Native American populations. The aforementioned studies investigating racial/ethnic minorities did not report any individuals identifying as two or more races; future studies should improve identification of race/ethnicity to include bi-racial participants.

2.3 Validity of ADP

Validity within Multi-Ethnic Samples

In a bi-racial sample of 25 white and 39 black males, race did not affect the accuracy of ADP compared to a 4C estimate (W: r=0.59, SEE =5.3%; B: r=0.76, SEE=4.7%). However, ADP demonstrated poor validity and underestimated %fat for both races. In a smaller sample of white (n=39) and black (n=3) females, ADP %fat demonstrated good validity with a 4C model (r^2=0.92, SEE=2.68%) [34]. Several investigations have evaluated the validity of ADP using DXA as the criterion. A study in a multiethnic sample of adults and children (13% minority; South Asian: n = 10; East Asian: n =3; African American: n=4; European American: n = 109) demonstrated good agreement between methods for adults (MD=1.6 ± 3.6 %, r^2 = 0.86, and root mean square error = 3.7 %) [24]. In a sample of overweight/obese females (White: n=17; Black: n =7), ADP FFM and %fat estimates were not significantly different than DXA measures (FFM: MD= 0.98 ± 2.92 kg, r = 0.90; %fat: MD=1.56 ± 3.75 %) [154]. An investigation of white (n=88.6%) and Asian/Asian Americans (n=11.4%) determined ADP %fat utilizing the Siri and Brozek equations were significantly different compared to DXA; however differences varied based on BMI category (Underweight: MD = 7.3 %, Normal: MD = 2.4 %; Overweight: MD = -1.48 %). In multi-ethnic populations, the validity of ADP is variable depending on the level of body fat of the population, the body density equation selected and criterion method utilized. Future evaluation of ADP should investigate validity compared to a multi-compartment model criterion in samples including all racial/ethnic minorities across BMI categories.

Validity by Race/Ethnicity

In 37 Mexican males and females (≥ 60 yrs), ADP %fat was not significantly different than a 3C criterion and had excellent validity (r^2= 0.97, SEE = 1.39%), however when evaluated by sex, males had significantly more individual variability (Limits of Agreement [LOA] = -4.4 – 2.5%) compared to females (LOA = -3.2 – 1.13%)[6]. Similarly, in 202 older Mexican adults (60-89 yrs), ADP FM estimates had very good validity compared to a 4C (r^2=0.93, SEE = 2.3 kg) [74]. A study of 30 black males 19-45 yrs determined ADP had good validity for body density estimates compared to hydrostatic weighing (r=0.91, SEE= 0.00721 g/cc), and very good validity for %fat measures compared to DXA ($r^{2=}$0.86, SEE = 2.84%) with ADP slightly overestimating %fat [141]. A large study of 445 Singaporean adults (91% Chinese ethnicity) found ADP to significantly underestimate %fat compared to DXA (MD = 3.9 %), however the methods were strongly correlated once adjusted for age, ethnicity and BMI (r=0.93) [13]. An investigation of 50 Japanese men demonstrated similar %fat estimates between ADP and DXA at baseline (MD = -0.4 ± 2.8%; r^2=0.63, SEE = 2.62%) and following a diet or exercise intervention (MD = 0.3 ± 2.9%; r^2=0.72, SEE = 2.92%), indicating ADP and DXA tracked body composition changes similarly following an intervention [113]. Very few studies have investigated race-specific validity of ADP in minority populations residing in the U.S. Additionally, to our knowledge no studies have investigated the validity of ADP in Native Americans or Pacific Islanders. Future studies should aim to evaluate race-specific validity of ADP compared to a multi-compartment criterion including both male and female minority adults residing in the U.S.

2.4 Validity of Bioelectrical Impedance Analysis

Several investigations have evaluated the validity of bioelectrical impedance devices. Primarily, studies have assessed the validity of regression equations created in large populations [i.e. Segal et al. [118] and Lukaski et al. [73]] for use in special populations including various races [15,129], elderly [41], children [46,124] overweight/obesity [106] and diseased states [35]. Initial studies validated BIA FFM and %fat utilizing HW as the criterion method, however more recent investigations have used DXA or a multi-compartment criterion. Bioimpedance estimations of total body water (TBW) and extracellular fluid (ECF) are validated against isotope dilution as the criterion [8,115] .

Validity within Multi-Ethnic Samples

A large study in Native American (n=247), Hispanic (n=111), and white (n=244) adults (18-72 yrs) evaluated the validity of previously published BIA equations for estimates of FFM and reported SEE = 2.22 – 5.21 kg, TE = 2.28 – 7.23 kg, r^2=0.73 – 0.89 compared to the HW criterion. A recent investigation utilizing a 4C model criterion evaluated an 8-electrode, multi-frequency BIA device (Seca Medical) regression equation in a multi-ethnic U.S. population (n=130; Hispanic, Asian, black and white) and reported pure error (PE) between 1.9-2.2 kg FFM and PE = 1.3-1.7 kg TBW. A study in 100 children and young adults (8-21 yrs) residing in the West Indian region (Afro-Jamaican, Asian and European ancestry) observed BIA (RJL Systems) %fat estimations to be acceptable compared to HW (r^2= 0.77, SEE = 3.7%) using manufacturer equations. More recent investigations have aimed to establish and validate regression equations in multi-ethnic (black, white, Hispanic, two or more races, Pacific Islander, Asian) samples of adolescents [46,124] and adults [127] and found including race as a predictor variable improved accuracy. However, a consensus on the most appropriate regression equation to minimize mean and individual error has not been established.

Validity by Race/Ethnicity

Several studies have investigated the validity of BIA in Asian populations including Chinese, Indonesian, Malay, Indian, Singaporean Chinese and Japanese participants. In 45 Indonesian adults, BIA demonstrated moderate to strong correlations (r = 0.63-0.97) and large %fat MD (4.8 - 8.0 %) when compared to a 3C model [62]. Additionally, in 298 Asian adults (Singaporean Chinese, Malay, Indian), BIA demonstrated fair validity (r = 0.87; SEE = 4.5%) compared to a 4C criterion [28]. A study in 162 Indian adults males investigating the validity of %fat measured by leg-to-leg BIA (Beurer BF 60) and handheld BIA (Omron) found strong correlations (r = 0.741 – 0.817) to DXA measures with no significant difference between the leg BIA estimate (MD = 0.72 %) to DXA, but a significant difference for handheld estimates (MD = 4.44 %) [137]. A larger difference was observed in a study of 200 Indian adults between BIA and DXA %fat values depending on the race specific equation utilized (MD = 5.4 - 8.3 %); both the

Caucasian and Asian equation underestimated %fat [90]. Studies that have created BIA regression equations in Chinese and Southeast Asian populations have determined excellent validity for TBW (SEE = 1.6 kg) when cross validated against an isotope dilution criterion method [48] and for FFM (r^2=0.99, RMSE = 0.133) validated against DXA. Similarly to multi-ethnic populations, incorporating race specific equations is important for valid estimates in Asian populations, but a consensus on the most accurate method may depend upon the country of origin and type of device used (i.e. handheld vs. leg-to-leg).

Previous investigations assessing the validity in individuals of African descent, have used a variety of criterion methods limiting translation. A previous study in black males (n=37) investigating BIA, using the Segal [118] equation, determined FFM was predicted accurately (r = 0.97, SEE = 2.1 kg) compared HW. Another investigation of 30 black males utilizing BIA manufacturer equations (RJL Systems) determined BIA was not valid for %fat compared to HW (r = 0.57, SEE = 5.9%, TE = 9.4%) [131]. More recently, a study including 250 North African adults cross validated a newly created regression equation, and previously published equations compared to isotope dilution, and reported variable error between equations for estimates of FFM (PE = 2.46 – 4.10 kg, LOA: -8.71 – 7.03 kg) and TBW (PE = 1.81 – 3.20 kg, LOA: -6.25 – 7.11 kg) [3]. In a similar investigation, five BIA equation estimates of %fat were cross validated with DXA estimates in a sample of 74 African American females and found poor validity for all equations (SEE = 4.20 – 4.70 %, r^2 = 0.39 - 0.52) [70]. Bioelectrical impedance estimates in black participants have demonstrated poor validity, however, further investigations assessing validity compared to a multi-compartment criterion has not yet been explored.

In Hispanic populations, validity of BIA estimates of body composition have not been thoroughly evaluated. A study investigating BIA estimates of FFM utilizing the Lukaski [73] equation determined a significant difference in Hispanic females (n=14, MD = -3.4 ± 2.6 kg, r = 0.89), but not males (n=70, MD = 0.54 ± 3.4 kg, r = 0.89) compared to the DXA criterion; this could also be influenced by the small female sample size. In 31 Hispanic females (n=22 were 100% Hispanic), several BIA equations were evaluated and demonstrated very good to excellent validity for FFM (SEE = 1.4 – 2.0 kg; r^2 = 0.76 – 0.90). Previous investigations did not use race-specific equations for Hispanic participants, therefore a study in 155 males and females from Mexico (20-50 yrs) created and cross validated a regression equation and found BIA FFM demonstrated good validity (r^2 = 0.97, PE = 2.96 kg, MD = 0.87 ± 2.84 kg) compared to ADP. Similar to other ethnicities, current literature in Hispanic individuals suggests BIA race-specific equations should be validated against a multi-compartment model.

Validity of BIA in Native American participants has not been evaluated recently. Rising et al. (1991) evaluated the validity of BIA FFM estimates using manufacturer software (SEE = 6.89 kg, r = 0.70) and a newly created equation in Native Americans (SEE = 3.22 kg, r = 0.92) and determined the race-specific equation improved validity from poor to acceptable compared to HW. A follow up study in Native American females determined race-specific and general BIA equations overestimated FFM (TE = 2.00 – 4.86 kg, SEE = 1.69 – 2.8 kg, r = 0.82 – 0.94) compared to a multi-compartment criterion. Studies investigating the validity of BIA in individuals who identify as two or more races and Pacific Islander have not been studied separately in adult populations, and therefore future research should include these two understudied racial/ethnic categories.

2.5 Validity of Bioelectrical Impedance Spectroscopy (BIS)

Few studies have investigated the validity of BIS body composition measures in minority populations. A study evaluating black, white and Hispanic adults (n=150) reported that two tetrapolar BIS devices (Inbody 320 and Inbody 770) demonstrated significant mean differences in females (MD= 2.99 %), but not males (MD = 0.36 %), and poor validity compared to a 4C criterion (TE = 5.0 – 5.5%, SEE = 4.8 – 5.2 %, r = 0.84 – 0.89) [45]. A study in African American college-age adults (n=143) showed BIS estimations were strongly correlated for FFM (r = 0.911 – 0.918) and %fat (r = 0.717 – 0.871) to ADP, however additional validity statistics were not reported [153]. Two studies evaluating multi-ethnic populations investigated the validity of BIS compared to isotope dilution, magnetic resonance imaging (MRI), [59] and DXA [84] in hemodialysis patients; thus , generalizability to healthy populations is limited. Future studies should evaluate the validity of BIS measures of TBW, FFM, and %fat in minority populations compared to criterion methods, eliminating the need for population-specific BIA equations.

2.6 Bioelectrical Impedance Segmental Measures

In addition to total body water, multi-frequency bioelectrical impedance devices can be utilized to estimate segmental (i.e. trunk, limb, VAT) tissue mass. However, age-related sarcopenia and connection tissue infiltration [158], segment-specific resistivity [162], BMI [119] and body position (standing vs. supine) [163] each may influence segmental extracellular fluid measures, subsequently affecting the validity of FFM estimations. Therefore, the aforementioned factors should be considered when evaluating segmental tissue mass. Proprietary BIA regression equations (seca mBCA) created in Caucasian participants demonstrated good validity for leg (MD = -0.54 – 0.97 kg) and arm (MD = -1.54 - -0.57 kg) lean soft tissue (LST) in a multi-ethnic (African American, Hispanic, Asian) sample compared to DXA and MRI [14]. In a study of 45 collegiate athletes, no racial data was reported, however, strong correlations (r = 0.82 – 0.89) and low error values (TE = 0.40-1.61 kg; SEE = 0.38 – 1.51 kg) were reported for BIS segmental LST compared to DXA [33]. In Caucasian populations, segmental BIA estimations have shown a wide range of validity. In 484 older adults (Mean age > 60 yrs), arm (MD = 0-0.2 kg), leg (MD = 0.4 - 0.6 kg) and trunk (MD = 2.8 – 3.7 kg) LM were similar compared to DXA estimations [66]. Conversely, a study in 17 females reported that BIA prediction of FFM had high relative error for the arm (15.9-16.9%), trunk (11.7-12.9%) and leg (11.9-12.7%) [16]. In a study of resistance-trained males, BIA estimated lower limb and trunk LM compared DXA; however, BIA was found to be acceptable for tracking changes following 10 weeks of resistance training, although BIA was less sensitive than DXA. In addition to segmental LST, BIA and BIS have been used to estimate thigh muscle volume and thigh cross sectional area in Caucasian [126] and Japanese [157] populations demonstrating variable results. Future research should further investigate the validity of segmental measures of LST, muscle volume and size in diverse samples.

2.7 Introduction Part 2: Muscle Characteristics

Muscle characteristics, specifically muscle size, quality, and architecture, have been shown to be related to muscle strength [19,37], muscle power [65], and cardiovascular performance [20]. Furthermore, increased intramuscular fat content may lead to changes in lipid metabolism and has been shown to be related to insulin resistance and the development of type II diabetes [121]. Ultrasonography, which has been validated for measures of muscle size and quality against computed tomography (CT), MRI, and muscle biopsies [4,97,112], has become a popular, time-efficient method for assessing muscle characteristics. In addition to investigating the relationship of muscle characteristics to functional performance, investigations utilizing ultrasonography have primarily focused on the effects of aging [38,44,132], obesity [91], and the acute and chronic response to training, particularly resistance training [19,75,103]. Similar to available body composition literature, race and ethnicity are not consistently reported in muscle characteristics data. Studies predominately have included a single racial/ethnic cohort [57,61,87], and not a multi-ethnic sample. Therefore, due to differences in ultrasound settings and testing conditions, systematically characterizing muscle characteristics in minority populations is challenging.

2.8 Muscle Size and Architecture

Ultrasound estimations of muscle size have most frequently utilized a single muscle cross sectional area (mCSA) measure. However, recent investigations have demonstrated that muscles do not uniformly adapt along the muscle length [151]. Therefore, a single mCSA measure may not provide an appropriate estimate of muscle size; a muscle volume (MV) estimate may more accurately represent muscle size. Muscle volume, commonly measured via MRI or CT, may be estimated more efficiently and with less radiation exposure by multiple panoramic ultrasound scans [92]. Few investigations have evaluated MV in ethnically diverse samples. An investigation utilizing MRI evaluated muscle mass in East Asian, Caucasian, and African American participants and observed differences in muscle size, with African Americans reporting the largest muscle size and Asians the smallest [42]. Similarly, a study of 79 adults between 50-85 years old observed a mean difference in MV measures via CT between Caucasian and African American participants, however no difference in functional power relative to muscle size was observed [31]. Muscle architecture, including pennation angle (PA) and fascicle length (FL) greatly influence the strength and force producing capacity of the muscle [40]. Differences in muscle size, but not force production and power, may suggest differences in muscle architecture exists between races/ethnicities. However, a study by Abe et al. [1] found no significance differences in PA, FL or muscle size between black and white collegiate football players. Studies in Japanese [57], Korean [61], and Hispanic

[87] populations have investigated effects of aging and training, but not characterized the racial/ethnic cohorts. Future investigations should more comprehensively investigate muscle volume and architecture in multi-ethnic populations and the resultant relationships to functional performance and health.

2.9 Muscle Quality

Muscle quality can be measured by a muscle biopsy to investigate fat and fibrous tissue infiltration or by clinical imaging (CT/MRI/ultrasound) utilizing a gray scale analysis (echo intensity [EI]) to estimate the amount of contractile versus non-contractile tissue within the muscle. An investigation using ultrasound to evaluate mCSA and EI among races, reported no significant difference in muscle size, but a difference in EI between black and white untrained, overweight/obese participants [78]. In a study using the same ultrasound techniques, the racial difference in muscle quality was no longer present in an athletic population [79]. Utilizing CT/MRI, fat infiltration has been investigated between black and white individuals [58,80,110], as well as in Asian individuals, specifically Indian Asian participants [32]. However, investigations including other minority populations are sparse. Katsiaras et al. [58] reported greater muscle density in elderly black males, but lower muscle density in elderly black females compared to white participants. Conversely, a study in older, sedentary males found greater fat infiltration, lower muscle quality and greater risk for development of Type II diabetes in Afro-Caribbean males compared to Caucasian males [80]. Increased fat accumulation in the muscle may represent metabolic changes in lipid metabolism including reduced fat oxidation and low basal ATP concentration [81]; reduced fat utilization will increase excess availability of fat or increase the uptake of lipid into the muscle [109]. The increase in intramuscular fat has been shown to be related to insulin insensitivity and the development of metabolic syndrome [47]. The prevalence of metabolic diseases in minority supports studying muscle quality among all racial/ethnic populations. Furthermore, few investigations utilize ultrasonography to evaluate racial/ethnic muscle quality, however, the ultrasound is more readily available and non-invasive compared to criterion techniques. Future studies should utilize the ultrasound to evaluate muscle quality more extensively in minorities.

2.10 Conclusion: Significance of Study

Although the minority population in the U.S. is increasing, racial/ethnic minorities are still under-represented in body composition and muscle physiology investigations. Due to the relationship between body composition, muscle characteristics, and cardiometabolic disease risk, it is vital to thoroughly investigate these components of health in minority populations. Potential differences in compartments of the body may contribute to inaccurate body composition assessment and lead to miscategorization of overweight/obesity and obesity-related disease risk. Results of this study may help inform researchers and practitioners of the best method for body composition measurements considering race/ethnicity and feasibility. Selecting the appropriate method may improve our ability to manage body composition effectively in racial and ethnic minorities. Accurately assessing body composition and characterizing body composition phenotype and muscle physiology in minority populations is essential for better interventions and disease prevention strategies.

CHAPTER III: METHODS

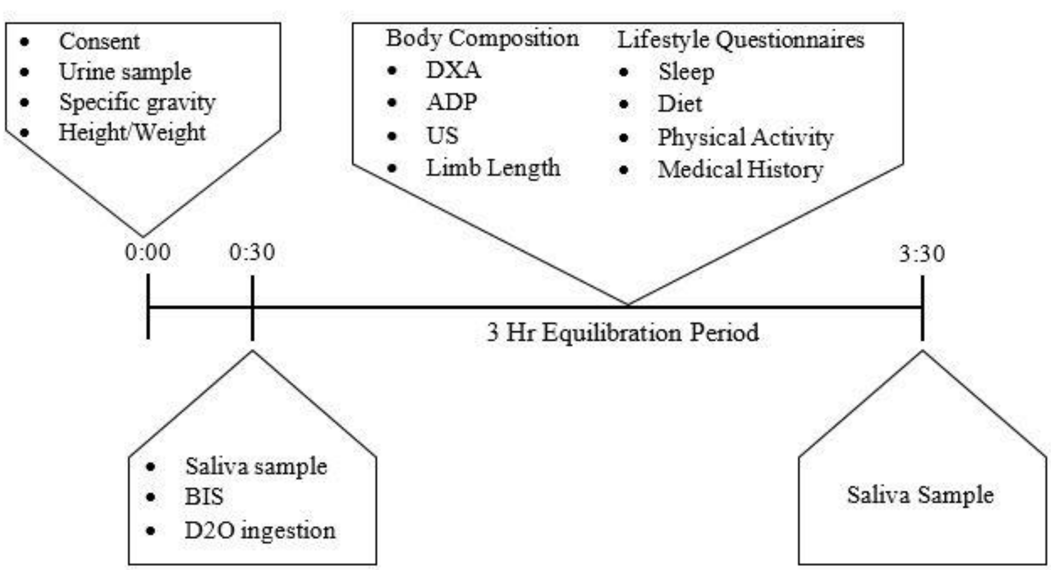
3.1 Participants

Between April 2019 and January 2020, participants were primarily recruited from three southeastern universities and the surrounding communities via flyers, social media, email, listerv announcements and presentations to organizations focused on minority population engagement. The proposed study enrolled 110 healthy males (n=49) and females (n=61). Participants were stratified by race/ethnicity as follows: 22 black/African American, 22 white/European descent, 22 Asian, 22 Hispanic/Latino/a, 1 Native American/Native Alaskan, 21 Two or more races (incl. Hispanic and White [n=11], Hispanic and Black [n=1], Black and White [n=6], Black and Asian [n=3]). Participants were eligible to participate if they were between the ages of 18-45 years and had a body mass index of 18.5-34.9 kg/m^2. Potential participants were excluded if they: 1) were currently pregnant or planned to become pregnant, 2) had gained or lost greater than 3 kg in previous two months, 3) had been diagnosed with a musculoskeletal disease; 4) had been diagnosed with a musculoskeletal injury limiting daily activities in the previous 3 months, 5) participated in 7 days per week of resistance or aerobic training, 6) had used anabolic steroids in previous year, 7) had a self-identified or clinically diagnosed eating disorder, 8) had been diagnosed with a disease that may result in significant changes in total body water (i.e. renal disease) or weight status (i.e. thyroid abnormalities).

3.2 Experimental Design

Participants were asked to report to the laboratory for a single testing session (Figure 1). Participants arrived euhydrated and a minimum of 12 hours fasted including having abstained from caffeine, alcohol and tobacco and from abstaining from strenuous exercise for a minimum of 24 hours. Prior to testing, all participants signed a consent form approved by the University's Institutional Review Board for the protection of human subjects. Following enrollment, participants provided a urine sample to assess hydration by urine specific gravity and a saliva sample for baseline total body water (TBW). A research technician measured height to the 0.1 cm using a portable stadiometer (Perspective Enterprises, Portage, MI, USA) and blood pressure using a standard automated cuff. Bioelectrical impedance device measurements were competed followed by ingestion of deuterium oxide (D_2O). Anthropometrics, body composition and muscle characteristics assessments including dual-energy x-ray absorptiometry (DXA), air displacement plethysmography (ADP), and brightness (B)-mode ultrasound (US) were conducted. Participants completed a series of lifestyle questionnaires including health history, sleep, diet and physical activity. Three hours following ingestion of D_2O, participants provided a second saliva sample for analysis of TBW.

Figure 1. Experimental design.



DXA = dual energy X-ray absorptiometry; ADP = air displacement plethysmography; US = ultrasound; BIS = bioelectrical impedance spectroscopy; D_2O = deuterium dilution

3.4 A**nthropometrics**

Waist and hip circumferences were measured by placing a measuring tape with a Gulick attachment around the trunk at the top of the iliac crest and the widest portion of the buttocks, respectively. Participants stood with feet together and body weight evenly distributed on both feet. For waist circumference, measurements were taken at the end of a normal expiration. The measuring tape was held in the horizontal plane, parallel to the floor and taut against the skin, without causing compression. Limb length measures were taken on the right side of the body. Participants laid supine with the right leg and arm fully extended. With the arm in a pronated position, a measuring tape was used to measure the arm length from the acromion process to the radial styloid process. Leg length was measured from the greater trochanter to the lateral malleolus. Upper leg length was measured from the greater trochanter to the lateral epicondyle. Measurements were recorded to the nearest 0.1 cm.

3.5 Body Composition

Body composition was assessed by nine existing body composition models, including three 4-compartment models (4C), three 3C models, and three 2C models. Models 1-3 were completed by using a 4-Compartment (4C) model described by Wang et al. (2002) [Equation 1] to determine fat mass (FM).

1) $\mathrm{FM\ (kg)} = 2.748\ (\mathrm{BV}) - 0.699\ (\mathrm{TBW}) + 1.129\ (\mathrm{Mo}) - 2.051\ (\mathrm{BM})$

where BV is body volume (L), TBW is total body water (L), Mo is total body bone mineral (kg) and BM is body mass (kg). For the Criterion 4C model, total body water was assessed with deuterium dilution, BV was estimated by air displacement plethysmography (ADP), and Mo was calculated from a total bone mineral content (BMC) measure estimated by a full body DXA scan (Mo = BMC × 1.0436). Models 1-3 are described in Table 3.

Models 4-5 (Table 4) was assessed utilizing the Siri 3-Compartment (3C) model presented by Wang et al. 1998 [Equation 2] to determine Fat mass (FM).

$$2)\ \text{FM (kg)} = 2.118(\text{BV}) - 0.780\ (\text{TBW}) - 1.351\ (\text{BM})$$

where BV is body volume, TBW is total body water and BM is body mass.

For models 1-5, Equations 3 and 4 were used to estimate body fat percentage (%fat) and lean mass (LM) following calculation of FM.

$$4)\ \%\text{fat} = (\frac{\text{FM}}{BM})\ \text{x}\ 100$$

$$5)\ \text{LM (kg)} = \text{BM} - \text{FM}$$

Models 6-9 estimated %fat, FM, and LM from single device measures: ADP, BIS_1 (SFB7 Impedimed), BIS_2 (Inbody 770), DXA.

Dual-Energy X-ray Absorptiometry

For each participant, a trained technician performed and analyzed a full body DXA (GE Lunar iDXA, Madison, WI, USA) scan to determine LST, FM, BMC, %fat, regional and segmental LST, FM, visceral adipose tissue (VAT), android/gynoid distribution and bone mineral density (BMD). Prior to testing, participants were asked to remove all metal, thick clothing, and heavy plastic to reduce interference with the scan. Birth date, height, weight, and race were entered into the DXA software. Participants were positioned supine in the center of the scanning table and instructed to remain still and breathe normally for the duration of the scan.

The regions-of-interest were automatically set by manufacturer software and then manually adjusted by the DXA technician.

Bioelectrical Impedance Spectroscopy

Two multi-frequency bioelectrical impedance devices (BIS_1: SFB7 ImpediMed, Queensland, Australia [10 - 500 kHz]; BIS_2: InBody 770; Biospace Co., Seoul, Korea [1-1000 kHz]) were used to assess TBW, extracellular fluid (ECF), intracellular fluid (ICF) and segmental water measures (arms, legs). For BIS_1, participants laid supine on a table with arms separated from the torso and legs separated. Prior to testing, height, weight, age, and sex were entered into the device, and each electrode site was cleaned with an alcohol wipe. Two electrodes were placed 5 cm apart on the dorsal side of the right wrist and hand, and two electrodes were placed on the dorsal side of the right ankle and foot for total body measures. For arm segmental measures, two electrodes were placed 5 cm apart on the right acromion and upper arm and two electrodes were placed 5 cm apart on the dorsal side of the right wrist and hand. For leg segmental measures, two electrodes were placed 5 cm part on the dorsal side of the right ankle and foot and two electrodes were placed 5 cm apart 10 cm distally from anterior iliac spine. For upper leg measures, the two distal electrodes were placed 5 cm apart 10 cm proximally from the tibial tuberosity. Device default settings for resistivity coefficients, the hydration constant (0.732), body density (1.05 kg/L) and the body proportion constant (4.30) were used to estimate TBW and total body FFM and FM. Segmental ECF, ICF, and total water were estimated in liters as follows:

1) ECF = $\frac{1}{1000} p_E (\frac{L^2}{R_E})$

2) ICF = $\frac{1}{1000} p_I (\frac{L^2}{R_I})$

3) ECF + ICF = TW

where p_I is the limb specific intracellular resistivity (Leg: 281 Ω cm for males and females; Arms: 191 Ω cm for females and 194 Ω cm for males), p_E is the limb specific extracellular resistivity (Leg: 98 Ω cm for males and 99 Ω cm females; Arms: 67 Ω cm for females and males)[162], L is length of segment between sensing electrodes measured in cm, R_E is measured extracellular resistance and R_I is measured intracellular resistance. FFM was estimated from total water of each segment using the hydration constant: FFM (kg) = Segmental TW (L) /0.732.

For BIS_2, participants were asked to stand upright for 5 minutes prior to measurement of TBW and segmental water. Participants stood barefoot on the device with their soles in contact with the foot electrodes with legs separated and were instructed to grasp the device handles with their palm, fingers and thumb making contact with the hand electrodes. Arms were raised to separate from the torso and participants were asked to stand still during measurement. Device software automatically estimated total body water, FFM and FM, and segmental water and FFM.

Deuterium Dilution

Total body water was measured by a criterion 3-hour deuterium (D_2O) dilution protocol in accordance with the International Atomic Energy Agency guidelines for isotope dilution. Participants provided a baseline saliva sample. Each participant ingested tap water with a dose of D_2O calculated to ensure an excess of 0.05 g ^2H per kilogram of body mass followed by 50 mL of tap water. The exact dose of D_2O was recorded for each participant. During the 3-hour equilibration period, participants could have up to 200 mL of water. The volume consumed by each participant was recorded. Three hours following ingestion, participants provided a post saliva sample. Saliva samples were stored in -20°C freezer until batch analysis was completed in triplicate via isotope-ratio mass spectroscopy at the University of Wisconsin Isotope Ratio Mass Spectrometry Laboratory. The baseline and D_2O-enriched saliva samples were used to calculate TBW including the correction factor for nonaqueous exchange of D_2O [115].

Air Displacement Plethysmography

Prior to each BV measurement, the device (BodPod®, COSMED USA, Inc., Concord, CA, USA) was calibrated according to manufacturer guidelines. Participants were asked to wear a swim cap and tight-fitting clothing such as a bathing suit or compression shorts, and to remove all metal including jewelry, watches and glasses prior to measurement to reduce isothermal air. Body mass was measured to the nearest 0.01 kg using the software's corresponding scale (Tanita Inc., Tokyo, Japan). During the assessment, participants were seated in an upright position and asked to minimize movement. Body volume was measured by a minimum of two trials that were within 150 mL of each other. Thoracic gas volume was measured via manufacturer instructions. In the event measured thoracic gas volume could not be obtained in three trials (n=49), the value was estimated by the software's standard prediction equations. Previous investigations have reported no significant differences between predicted and measured lung volume in adults [24,77].

3.6 Muscle Characteristics

Muscle characteristics of the right vastus lateralis (VL) were assessed from panoramic and longitudinal images captured using a B-mode US (Logiq-e, GE Healthcare, Wisconsin, USA). The ultrasound settings (Frequency = 10, Gain = 46) were kept consistent to standardize measures; depth was adjusted to optimize the image. Prior to each scan, subjects were instructed to lay supine with the right leg extended and relaxed on the examination table. Three panoramic scans of the thigh were performed; a high-density foam pad will be strapped to the thigh at 25%, 50% and 75% of the distance of the VL. A wide-band linear array ultrasound transducer probe (GE: 12L-RS) was held perpendicular to the tissue and swept across the skin at equal pressure from the lateral VL border to medial fascia separation. For longitudinal scans, a still image was taken with the probe held perpendicularly to the tissue on the anterior midpoint of the thigh. The same technician performed each scan and reviewed the initial quality of images on the US monitor.

Muscle cross sectional area and EI were determined from the panoramic scans of the VL using Image-J software (National Institute of Health, USA, Version 1.37). Echo intensity was determined from the scan at 50% VL length (Depth = 6 cm for all scans) in the standard histogram function, which uses grayscale analysis of pixels ranging from 0 to 255. Prior to measuring mCSA and EI, each image was calibrated by measuring the number of pixels within a known distance of 1 cm. To measure mCSA and EI, as previously described by Cadore et al. [20], the same technician traced the outline of the VL for each subjects' scan along the fascia border as close as possible to capture only the muscle. A correction factor for subcutaneous thigh fat thickness, previously described by Young et al. [160], was used to account for the influence THfat has on EI measures: EI_C (a.u.) = $y_1 + (x * cf)$; where y_1 = raw EI, x =THfat, cf = 40.5278, and EI_C = corrected EI. Subcutaneous THfat was determined by a linear measure from the epidermal layer to the fascial border of the VL.

Muscle thickness (MT) was determined from the longitudinal scan by measuring the distance between the inferior border of the superficial aponeurosis and superior border of the deep aponeurosis. An additional panoramic scan was performed at the midpoint of the thigh along the fascicle plane ensuring inclusion of a visible fascicle. ImageJ software was used to measure the length of the visible fascicle (FL) and pennation angle (PA). Pennation angle was measured by determining the angle at the intersection between the superior border of the deep aponeurosis and a visible fascicle [7]. Muscle volume (MV) was estimated by the Cavalieri formula [92] utilizing the three panoramic scans as follows: $MV = \sum_n e_i \times mCSA_i$;where n = number of slices/scans, and e = distance between slices.

3.7 Demographics and Lifestyle Questionnaires

Participants were asked to self-report personal and parental demographic information and to complete 1) a health history questionnaire which included indices to assess sleep, physical activity and dietary habits and 2) the Perceived Stress Scale. Additionally, participants completed a 3-day dietary intake log to estimate total calories and macronutrients (carbohydrates, protein, fat). Participants were instructed to write down all food and beverage consumed during 2 weekdays and 1 weekend day.

3.8 Statistical Approach

Sample Size Determination

G*Power software was used to calculate sample size requirements (matched pairs t-test) to ensure statistical power of 0.8 at an alpha level of 0.05. A previous study by Bosy-Westphal et al. [15] assessed the validity of a bioelectrical impedance device compared to a 4C criterion model in a multi-ethnic sample. The reported mean difference between methods for measures of FFM was 0.8 ± 1.9 kg; the calculated effect size = 0.42, estimates 47 participants would be sufficient to power the current study. Analysis in each race separately found a mean difference in FFM of 0.70 ± 1.8 kg (Asian), 0.4 ± 1.80 kg (Hispanic) and 1.5 ± 1.7 kg (African American) with effect sizes between 0.22-0.88, which estimated a sample size between 13-161 for power of 0.8. Another investigation by Bosy-Westphal et al. [14] evaluated the validity of bioelectrical impedance analysis predictions of segmental lean soft tissue compared to DXA and found mean differences of -0.54 ± 1.13 kg (African American), 0.97 ± 0.79 kg (Asian) and 0.74 ± 1.08 kg (Hispanic) with effect sizes between 0.48-1.28, estimating 8-37 participants required for appropriate power.

Statistical Analysis

Aim 1: Mean, standard deviation, mean difference and confidence intervals were calculated for each model for the entire sample and separately within each racial/ethnic cohort. Total error (TE $=\sqrt{\sum[\text{predicted-actual}]^2/n}$; the average deviation of individual scores from the line of identity [50]), standard error of the estimate (SEE $=\sqrt{\sum[\text{predicted-actual}]}\bullet\sqrt{1-r^2}$; the degree of deviation of the individual data points around the line of best fit [50]), Pearson's correlation coefficients and linear regressions were completed to determine the agreement of each body composition model for estimates of %fat and FFM compared to the criterion. Subjective ratings were reported according to the Heyward and Wagner [50] validity subjective rating scale. Paired samples t-tests were also performed to evaluate the agreement between body composition models. To assess individual variability, Bland-Altman plots for % fat and FFM, were constructed and proportional bias was assessed by linear regression analyses for each body composition model.

Aim 2: The validity statistics performed in aim 1 were repeated for aim 2 to assess the agreement between the two bioelectrical impedance device estimates of total body water compared to deuterium dilution criterion for the entire sample and separately within each racial/ethnic cohort. Paired samples t-tests were also be performed to evaluate the difference between bioelectrical impedance estimates of total body water compared to deuterium dilution.

Aim 3: The validity statistics performed in aim 1, were repeated for aim 3 to assess the agreement between the bioelectrical impedance device estimates of segmental (arm, leg) FFM compared to DXA for the entire sample and separately within each racial/ethnic cohort. Paired samples t-tests were performed to evaluate the difference between bioelectrical impedance segmental FFM estimates compared to DXA segmental FFM. Mean ± SD, mean differences and confidence intervals were reported.

For the exploratory aim, separate one-way analysis of variance (ANOVA) tests and Chi^2 tests were conducted to assess diet (calories, protein, carbohydrates, fat, sugar), physical activity, stress, and socioeconomic status to identify potential cofounding variables (Appendix II). No differences were found to be significant, therefore no variables were included as covariates for subsequent analyses. One-way ANOVA tests were performed to compare racial/ethnic groups measures of body composition (fat distribution, VAT, BMD) and muscle characteristics (MV, mCSA, EI, PA, FL). When the ANOVA was found to be significant, Tukey HSD post-hoc analyses were performed to assess differences between groups.

All statistical analyses were performed using SPSS Version 21 Statistical Analysis. For aims 1-3, all analyses were assessed in the full multi-ethnic sample and in each race/ethnicity cohort separately. An alpha level of 0.05 was set a priori.

CHAPTER IV: MANUSCRIPT 1

Validation of body composition methods across multiple races and ethnicities

Introduction

Minority populations in the United States are at an increased risk for numerous chronic diseases including obesity and the related comorbidities[63,95,133]. Due to the relationship between body composition and cardiometabolic disease risk [11,86], it is vital to thoroughly investigate this component of health in minority populations. Body mass index (BMI) is widely used in clinical settings to assess obesity and the associated health risks. However, BMI is incapable of differentiating between the various components of body composition and may mischaracterize overweight and obesity, especially in racial/ethnic minorities due to slight physiological differences[27,53,138,139]. Multi-compartment models are considered the gold standard method for analysis of body composition[50,149], as they measure several constituents of the body such as total body water, bone mineral content, and soft tissue mineral content. A comprehensive study by Wang et al.[148] investigated the validity of 16 body composition models compared to the 6-compartment neutron activation model and found multi-compartment models that include total body water estimates demonstrated the lowest total error and highest reliability. A subsequent study[147] concluded the 4-compartment (4C) model has similar validity to a 6C model, with greater feasibility.

However, multi-compartment models require a minimum of two devices to measure additional compartments of the body and may not be the most feasible or practical technique.

Therefore, single-device two-compartment (2C) models (i.e. air displacement plethysmography [ADP], bioelectrical impedance analysis/spectroscopy [BIA/BIS]) and three-compartment (3C) models (i.e. dual-energy X-ray absorptiometry [DXA]) are more commonly utilized to estimate fat mass (FM) and fat free mass (FFM). As two-compartment models measure fewer body compartments, several assumptions must be met for accurate measures of body composition. BIA and BIS devices may be influenced by hydration, body proportion and fat distribution[36,49], while ADP may be influenced by fat free body density estimations[36]. Previous studies have observed racial/ethnic differences in trunk and limb length[139], FM and FFM distribution in the trunk and limbs[53], as well as differences in bone mineral content[138,139], potentially contributing to differences in fat free body density. Racial/ethnic variations in body compartments may influence the ability of body composition models to accurately assess body composition.

Investigations that have evaluated the validity of body composition models in racial/ethnic minorities are limited. Few studies have used a criterion multi-compartment model to determine validity[15,28,45]; the majority of studies include only one racial/ethnic category[51,131,137,156] and evaluate a single body composition technique[5,13,100] which limits our ability to compare validity across multiple devices and populations. To our knowledge, no studies have investigated the validity of a commonly utilized BIS device (Impedimed SFB7) in minority populations, even though this technology is frequently used to evaluate lymphedema in various populations. Furthermore, technology has advanced significantly since initial validity studies were conducted, and therefore conclusions are based on outdated models and software. Validity investigations evaluating up-to-date technology in a more diverse sample may improve our ability to select the appropriate method to accurately assess body composition in specific racial/ethnic populations. Therefore, the purpose of this study was to assess the validity of existing body composition models compared to a 4-compartment (4C) criterion model for measures of body fat percentage (%fat) and fat free mass (FFM) in a multi-ethnic sample stratified by race and ethnicity.

Materials and Methods

Participants

One hundred and thirty-four individuals were screened for eligibility (Figure 2). Following screening, 110 adults (55% Female; Table 1) enrolled in the present study. Participants were stratified by race/ethnicity in the following cohorts: African American/Black (AA; n=10 male; n=12 female), Caucasian/White (W; n=10 male; n=12 female), Asian (A; n=10 male; n=12 female), Hispanic (H; n=10 male; n=12 female), Native American (NA, n=1), and Multi-Racial [MR; n=9 male; n=12 female; incl. Hispanic and White (n=11), Hispanic and Black (n=1), Black and White (n=6), Black and Asian (n=3)]. The distribution of the sample by race, age, and body mass index (BMI; kg/m^2) is displayed in Table 2. Participants were excluded if their BMI was <18.5 or >39.99, if they were pregnant or planning to become pregnant, if they had gained or lost greater than 3 kg in previous two months, had been diagnosed with a musculoskeletal disease; had been diagnosed with a musculoskeletal injury limiting daily activities in the previous 3 months, actively participated in 7 days per week of resistance or aerobic training, had used anabolic steroids in previous year, had a self-identified or clinically diagnosed eating disorder, or had been diagnosed with a disease that may have resulted in significant changes in total body water (i.e. renal disease) or weight status (i.e. thyroid abnormalities). Prior to participation, all participants signed a consent form approved by the University's Biomedical Review Board for the protection of human subjects.

Experimental Design

Participants reported to the laboratory for a single testing session after a 12 hour fast including abstention from caffeine, alcohol and tobacco. (Figure 3). Participants refrained from strenuous exercise for a minimum of 24 hours prior to testing. Following enrollment, participants provided a urine sample to assess hydration by urine specific gravity (USG = 1.002-1.029) and a saliva sample for baseline total body water (TBW) estimates. A research technician measured height to the 0.1 cm using a portable stadiometer (Perspective Enterprises, Portage, MI, USA) and weight to the nearest 0.1 kg using a calibrated scale (Tanita Inc., Tokyo, Japan). Bioelectrical impedance device measurements were completed followed by ingestion of deuterium oxide (D_2O) for TBW measurement. The remaining anthropometric (limb length, waist and hip circumference) and body composition assessments (dual energy X-ray absorptiometry, air displacement plethysmography) were then completed. Three hours following ingestion of D_2O, participants provided a second saliva sample for analysis of TBW.

Anthropometrics

Waist and hip circumferences were measured by placing a measuring tape with a Gulick attachment around the trunk at the top of the iliac crest and the widest portion of the buttocks, respectively. Participants stood with feet together and body weight evenly distributed on both feet. Limb length measures were taken on the right side of the body. Participants laid supine with the right leg and arm fully extended. With the arm in a pronated position, a measuring tape was used to measure the arm length from the acromion process to the radial styloid process. Leg length was measured from the greater trochanter to the lateral malleolus. Measurements were recorded to the nearest 0.1 cm.

Body Composition

Body composition was assessed by nine existing body composition models, including three 4-compartment models (4C), three 3C models, and three 2C models. Models 1-3 were completed by using a 4-Compartment (4C) model described by Wang et al. (2002) [Equation 1] to determine fat mass (FM).

$$1)\ \text{FM (kg)} = 2.748\ (\text{BV}) - 0.699\ (\text{TBW}) + 1.129\ (\text{Mo}) - 2.051\ (\text{BM})$$

where BV is body volume (L), TBW is total body water (L), Mo is total body bone mineral (kg) and BM is body mass (kg). For the Criterion 4C model, total body water was assessed with deuterium dilution, BV was estimated by air displacement plethysmography (ADP), and Mo was calculated from a total bone mineral content (BMC) measure estimated by a full body DXA scan (Mo = BMC × 1.0436). Models 1-3 are described in Table 3.

Models 4-5 (Table 4) was assessed utilizing Siri 3-Compartment (3C) model presented by Wang et al. 1998 [Equation 2] to determine Fat mass (FM).

$$2)\ \text{FM (kg)} = 2.118(\text{BV}) - 0.780\ (\text{TBW}) - 1.351\ (\text{BM})$$

where BV is body volume, TBW is total body water and BM is body mass.

For models 1-5, Equations 3 and 4 were used to estimate body fat percentage (%fat) and lean mass (LM) following calculation of FM.

$$3)\ \%\text{fat} = \left(\frac{\text{FM}}{BM}\right) \text{x}\ 100$$

$$4)\ \text{LM (kg)} = \text{BM} - \text{FM}$$

Models 6-9 estimated %fat, FM, and LM from single device measures: ADP, BIS_1 (SFB7 Impedimed), IB (Inbody 770), DXA.

Dual-Energy X-ray Absorptiometry

For each participant, a trained technician performed and analyzed a full body DXA (GE Lunar iDXA, Madison, WI, USA; enCORE Software Version 16) scan to determine LST, FM, BMC, and %fat. Prior to testing, participants were asked to remove all metal, thick clothing, and heavy plastic to reduce interference with the scan. Birth date, height, weight, and race were entered into the DXA software. Participants were positioned supine in the center of the scanning table. Subjects were instructed to remain still and breathe normally for the duration of the scan. The regions-of-interest were manually adjusted by the DXA technician.

Deuterium Dilution

Total body water was measured by a criterion 3-hour deuterium (D_2O) dilution protocol in accordance with the International Atomic Energy Agency guidelines for isotope dilution. A research technician prepared a large batch of the D_2O solution composed of 5 L of tap water and 515 g of D_2O. Individual doses of 58 g, 67 g, 79 g, 95 g and 114 g were prepared to ensure an excess of 0.05 g ^2H per kilogram of body mass. Based on body mass and sex, participants consumed a dose of the D_2O solution after providing a 2 mL baseline saliva sample. The exact dose of D_2O was recorded to the nearest 0.01 g for each participant. During the 3-hour equilibration period, participants were allowed to have up to 250 mL of water. The volume consumed by each participant was recorded. Three hours following ingestion, participants provided a post saliva sample. Saliva samples were stored in -20°C freezer until batch analysis was completed in triplicate via isotope-ratio mass spectroscopy at the University of Wisconsin Isotope Ratio Mass Spectrometry Laboratory. The baseline and D_2O-enriched saliva samples were used to calculate TBW including the correction factor for nonaqueous exchange of D_2O [115].

Bioelectrical Impedance Spectroscopy

Two multi-frequency bioelectrical impedance devices (BIS: SFB7 ImpediMed, Queensland, Australia [10 - 500 kHz]; IB: InBody 770; Biospace Co., Seoul, Korea [1-1000 kHz]) were used to assess TBW. For BIS_1, participants laid supine on a table with arms separated from the torso and legs separated. Prior to testing, height, weight, age, and sex were entered into the device, and each electrode site was cleaned with an alcohol wipe. Two electrodes were placed 5 cm apart on the dorsal side of the right wrist and hand, and two electrodes were placed on the dorsal side of the right ankle and foot for total body measures. Device default settings for resistivity coefficients, the hydration constant (0.732), body density (1.05 kg/L) and the body proportion constant (4.30) were used to estimate TBW and total body FFM and FM.

For IB, participants were asked to stand upright for 5 minutes prior to measurement of TBW. Participants stood barefoot on the device with their soles in contact with the foot electrodes with legs separated and were instructed to grasp the device handles with their palm, fingers and thumb making contact with the hand electrodes. Arms were raised to separate from the torso and participants were asked to stand still during measurement. Device software automatically estimated total body water, FFM and FM.

Air Displacement Plethysmography

Prior to each BV measurement, the device (BodPod®, COSMED USA, Inc., Concord, CA, USA) was calibrated according to manufacturer guidelines. Participants were asked to wear a swim cap and tight-fitting clothing such as a bathing suit or compression shorts, and to remove all metal including jewelry, watches and glasses prior to measurement to reduce isothermal air. Body mass was measured to the nearest 0.01 kg using the software’s corresponding scale (Tanita Inc., Tokyo, Japan). During the assessment, participants were seated in an upright position and asked to minimize movement. Body volume was measured by a minimum of two trials that were within 150 mL of each other. Thoracic gas volume was measured via manufacturer instructions. In the event measured thoracic gas volume could not be obtained in three trials (n=49), the value was estimated by the software's standard prediction equations. Previous investigations have reported no significant differences between predicted and measured lung volume in adults [24,77].

Statistical Analysis

Mean, standard deviation, mean difference and confidence intervals were calculated for each model for the entire sample and within each racial/ethnic cohort. Total error (TE $=\sqrt{\sum[\text{predicted-actual}]^2/n}$), standard error of the estimate (SEE $=\sqrt{\sum[\text{predicted-actual}]}\bullet\sqrt{1-r^2}$), Pearson's correlation coefficients and linear regressions were completed to determine the agreement of each body composition model for estimates of %fat and FFM compared to the criterion. Subjective ratings were reported according to the Heyward and Wagner [50] validity subjective rating scale. Paired samples t-tests were also performed to evaluate the agreement between body composition models. To assess individual variability, Bland-Altman plots for % fat and FFM, were constructed and proportional bias was assessed by linear regression analyses for each body composition model. An alpha level of 0.05 was set a priori. Statistical analyses were performed using SPSS (IBM Corp, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY).

Results

Multi-Compartment Device Measures

For the total multi-ethnic sample, measures of %fat and FFM from multi-compartment models were all excellent to ideal (%fat: TE = 0.94 – 2.37 %, SEE = 0.39 – 1.99 %; FFM: TE =

0.72 – 1.78 kg; SEE = 0.30 – 1.62 kg), with the exception of the DXA-BV 4C model, which was good to fairly good for %fat (TE = 3.79 %; SEE = 3.50 %) and excellent to very good for FFM (TE = 2.49 kg; SEE = 1.62 kg). Of the multi-compartment models the lowest error was observed for the D_2O 3C, followed by the BIS 3C and 4C models, with DXA-BV 4C demonstrating the highest error. Simple regression analyses indicated all models had a significantly different slope (0.85 – 0.97) and intercept (1.61-2.60) compared to the reference line of identity (slope = 1, intercept = 0), except the BIS 4C intercept (0.88, $p=0.056$) (Figure 4A). All multi-compartment models demonstrated significantly different means ($p<0.01$) for %fat (24.1 – 26.4 %) and FFM (53.5 – 55.2 kg), compared to the criterion (%fat: 25.6 %; FFM: 54.1 kg). Bland-Altman plot and regression analyses (Figure 5A-D) demonstrated individual variability for the total sample was greatest for the DXA-BV 4C %fat measures (95% limits of agreement [LOA]: -8.3 - 5 .4 %) and smallest for the D_2O 3C model (LOA: -0.1 – 1.7 %). Proportional bias was present for all multi-compartment models ($p<0.05$).

When stratified by race/ethnicity (Table 5), according to TE for %fat, the D_2O 3C, BIS 4C BIS 3C models were very good to excellent for all races (A: 1.0 – 2.4%; AA: 0.9 – 2.4%; H: 1.0 – 2.4%; MR: 0.9 – 2.4%), with the highest error observed for Caucasian/White individuals (1.0 – 2.7%). Based on TE, the DXA-BV 4C model %fat estimates were fairly good to fair for Asian (4.2%) and Hispanic (4.3%) participants, but performed more accurately for African American (3.4%), Caucasian/White (3.5%) and Multi-racial (3.5%) individuals based on TE. However, BV estimates between DXA and ADP were ideal for all groups, with the only significant mean difference (0.44 L; $p=0.014$) observed between methods for African American/Black participants (Table 7).

For measures of FFM, all multi-compartment models provided valid results for all races/ethnicities (Table 5). For BIS 4C, D_2O 3C and BIS 3C, TE indicated results were excellent to ideal (A: 0.7 – 1.7 kg; AA: 0.7 – 1.8 kg; H: 0.7 – 1.7 kg; MR: 0.7 – 1.9 kg; W: 0.7 – 2.1 kg), but slightly less accurate for DXA-BV FFM (2.4 – 2.6 kg).

For %fat and FFM, mean differences (MD; $p<0.05$) were observed for all multi-compartment models compared to the criterion for Asian (%fat: 0.7 – 3.5%; FFM: -0.4 – 2.1 kg) and Hispanic individuals (%fat: 0.8 – 2.2%; FFM: -0.6 – 1.3 kg). For Multi-racial individuals, MD was significant ($p<0.01$) between the BIS 4C (%fat: -1.3%; FFM: 1.0 kg) and D_2O 3C estimates (%fat: 0.7%; FFM: -0.6 kg) compared to the criterion; for Caucasians, MD were significant for BIS4C, D_2O 3C and BIS 3C (%fat: -1.8 -1.1%; FFM: -0.6 – 1.4 kg; $p<0.05$). In the African American/Black sample, the D_2O 3C estimates demonstrated the only significant MD (%fat: 0.8%; FFM: -0.6 kg; $p<0.001$). The BIS 4C, DXA-BV 4C, and BIS 3C models all underestimated %fat values (~1-2%) and overestimated FFM (~1 kg); with the opposite relationship observed for the D_2O 3C model (Table 7).

Single Device Measures

In the total sample, for the single device models, %fat measures were very good to excellent for DXA, ADP and IB (TE = 2.71%, 2.52%, 2.89%; SEE = 1.53%, 1.55%, 2.87%), and fairly good for BIS (TE = 4.12%, SEE = 4.03%). For FFM, DXA, ADP, and IB estimates were excellent to ideal (TE= 1.80 kg, 1.77 kg, 2.15 kg; SEE = 1.21 kg, 1.19 kg, 2.14 kg) and BIS estimates were good to very good (TE = 3.12 kg, SEE = 3.10 kg). For the total sample, ADP and DXA has the lowest error, followed by the IB and BIS demonstrated the highest error. Simple regression analyses indicated all models had a significantly different slope (0.73 – 1.04) and intercept (1.61-2.60) compared to the reference line of identity, except the IB intercept (1.4, p=0.074) (Figure 4B). DXA and ADP estimates of %fat (DXA: 27.7 %; ADP: 23.7 %) and FFM (DXA: 52.8 kg; ADP: 55.4 kg) were significantly different (p<0.01) compared to the criterion (%fat: 25.6 %; FFM: 54.1 kg), while IB (%fat: 25.2 %, p=0.200; FFM: 54.4 kg, p=0.154) and BIS (%fat: 25.1, p=0.221; FFM: 54.6 kg, p=0.083) means were not significantly different from the criterion. Bland-Altman plot and regression analyses (Figure 6A-D) demonstrated individual variability for the total sample was greatest for the BIS %fat measures (LOA: -8.5 – 7.6 %) and smallest for the DXA (LOA: -1.2 – 5.4 %) and ADP (LOA: -5.2 – 1.4 %) devices. Proportional bias was present for BIS, DXA, and ADP (p<0.01), but not for the IB (p=0.449).

When stratified by individual race/ethnicity, according to TE, %fat estimates for DXA were very good to excellent for all races/ethnicities (Table 6). Compared to the Caucasian/White sample (2.7%), DXA TE values were similar between Asian (2.6%), African American/Black (2.6%) and Hispanic (2.8%) participants, but less valid for Multi-Racial (2.9%) individuals. SEE values (1.1 – 1.9%) indicated DXA estimates were ideal for all races/ethnicities. ADP estimates were ideal to very good for all races/ethnicities according to TE (A: 2.6%; AA: 2.4%; H: 2.6%; MR: 2.7%; W: 2.3%).

Based on TE, the BIS device estimates were fairly good to poor for African American/Black (4.6%), Caucasian/White (4.9%) and Multi-racial (4.3%) samples, but performed more accurately for Asian (3.1%) and Hispanic (3.5%) participants. The IB produced more valid %fat estimates compared to the BIS for all races/ethnicities, except Asian which demonstrated similar results between devices (Table 6). IB measures were very good to excellent for Hispanic (2.4%) and Multi-racial (2.8%) participants; slightly less accurate results were observed for Asian (3.1%), African American/Black (3.2%), and Caucasian/White (3.4%) individuals.

For FFM measures, the DXA and ADP produced excellent to ideal results for all races/ethnicities (TE = 1.6 – 2.1 kg; SEE = 0.76 – 1.6 kg). Similarly to %fat estimates, the IB FFM measures were more valid compared to the BIS ranging from good (W: TE = 2.5 kg) to ideal (H: TE = 1.8 kg); BIS estimates were between fairly good (TE = AA: 3.4 kg; MR: TE = 3.4 kg; W: TE = 3.7 kg) to very/good excellent (A: TE = 2.2 kg; H: TE = 2.7 kg) (Table 8).

Although TE/SEE results varied by race/ethnicity, all groups demonstrated significant mean differences ($p<0.05$) for estimates from the DXA (%fat: 1.5 – 2.4%; FFM: -1.5 - -0.7 kg) and ADP (%fat: -2.2 - -1.2%; FFM: 0.7 – 1.7 kg) compared to the criterion (Table 6). For all races/ethnicities, there were no significant differences in %fat and FFM for the BIS ($p=0.126$-0.957) and IB ($p=0.078$–0.994) compared to the criterion, except for Asian individuals; the BIS was significantly different compared to the criterion (%fat: -1.4%; FFM: 1.1 kg; $p<0.05$) (Table 6). For %fat and FFM, ADP and DXA produced the most accurate estimates, followed by the IB and then BIS. For each race/ethnicity, DXA overestimated %fat (~2%) and underestimated FFM (~1-2 kg), with the opposite relationship observed for ADP (underestimated %fat ~1-2%, overestimated FFM ~1 kg).

Discussion

There are few investigations evaluating the validity of up-to-date body composition technology across diverse populations, even though the minority population in the U.S. is increasing. Identifying the most valid body composition models and devices may improve the ability of investigators and clinicians to select the appropriate method to accurately assess body composition in specific racial/ethnic populations. For multi-compartment models, the BIS 4C, BIS 3C and D_2O 3C models demonstrated excellent to ideal agreement with the 4C criterion for measures of %fat and FFM based on TE, SEE and R^2 values. The DXA-BV 4C model estimates demonstrated lower agreement with the criterion compared to other models, but were still good to very good for %fat and very good to excellent for FFM. Results from the present study indicate multi-compartment models may provide more accurate estimates compared to single device models. However, further research should validate the DXA-BV 4C model in multi-ethnic samples before its use as a criterion for validation studies, especially in Asian and Hispanic participants.

Previous literature evaluating body composition validity in minority populations and multi-ethnic samples have primarily focused on single device (DXA, ADP, BIA/BIS) estimates of %fat, FM and FFM [6,15,23,113]. There is limited data comparing alternative multi-compartment models to a criterion, even though many studies utilize the alternative multi-compartment models[45,62] or the DXA as the criterion to validate single device measures[35,137,142]. Wang et al.[148] evaluated several multi-compartment model estimates of fat mass in a multi-ethnic sample compared to 6C criterion and found disparate SEE values between 0.22 - 4.19 kg and MD between 0.78 – 4.75 kg. The models that were determined to be the most accurate incorporated a measurement of TBW and demonstrated SEE (0.97 – 1.08 kg) and MD (0.78 – 1.02 kg) values comparable to the SEE and MD observed in the present study for FFM (SEE = 0.30 – 1.62 kg; MD = -0.6 – 1.1 kg). The multi-compartment models in the present study performed similarly across each race/ethnicity and demonstrated excellent agreement, except %fat estimates from the DXA-BV 4C model in Asian and Hispanic individuals, which demonstrated fair agreement. The errors observed for the DXA-BV 4C model may be related to BIS TBW measures, as BV estimates between DXA and ADP were ideal for Asian and Hispanic groups (MD: -0.24 – 0.07 L; TE: 0.65 – 0.83 L; SEE: 0.27 – 0.43 L). Bland Altman analyses demonstrated the D_2O 3C model had the lowest individual variability and the DXA-BV 4C had the greatest. The small

MD, narrow LOA and ideal TE/SEE values from the D_2O 3C model was likely a result of the large contribution of D_2O TBW estimates to both the D_2O 3C model and the 4C criterion. Proportional bias was observed for all multi-compartment models; for each model, individuals with greater %fat were significantly underestimated.

For the single device body composition measures, the DXA and ADP demonstrated very good to excellent agreement for %fat, and excellent to ideal agreement for FFM measures for the multi-ethnic sample and in each race/ethnicity. However, both device estimates of %fat and FFM were significantly different than the criterion; the DXA overestimated %fat (~2%) and underestimated FFM (~1-2 kg), while ADP underestimated %fat (~1-2%) and overestimated FFM (~1 kg). A recent study evaluating the reliability of body composition devices in young adults reported standard error of the measurement values for %fat and FFM of 0.45%, 0.72 kg, respectively for DXA and 1.28% and 1.30 kg, respectively for ADP [117]. These results indicate the mean differences observed in the present study are beyond the sensitivity of the device, suggesting that investigators should cautiously interpret results and recognize the likelihood of a true over or underestimation of measures. The IB and BIS, %fat and FFM estimates were less accurate in all races/ethnicities. However, IB did not display any proportional bias, suggesting it would perform similarly across a broad sample of individuals. Overall, devices did not perform better in Caucasian/White individuals, compared to other races/ethnicities. Validation studies that have evaluated the DXA, in multi-ethnic samples have demonstrated SEE values of 1.6 kg for FFM [136] measures and 2.8 % for %fat [102], similar to the present study results (1.8 kg, 2.7 %, respectively). In individual races/ethnicities, previous literature has reported smaller MD (-0.2 - -0.3 %fat) [22,140] and similar TE (2.39 %) [140] for African American/Black participants and slightly larger MD (2.1 – 4.2 %) [25] in Asian individuals compared to the present study. Data evaluating

DXA validity in Hispanic and Multi-racial individuals are lacking. For ADP, studies investigating multi-ethnic samples have reported SEE values of 2.7 % [34] and MD ranging from -1.8 % - 2.4 % for %fat [34,71,154], similar to the present study (SEE = 1.55 %; MD = 1.9%). However, these studies had very small minority representation (7 – 30% of sample) and only included African American/Black and Asian participants. For Hispanic cohorts, to our knowledge, validity studies have only included Mexican individuals and found SEE results (%fat: -1.4 %; FM: 2.3 kg) [5,6], similar to our findings (SEE= 1.2 %,), but smaller MD (%fat: -0.99 %) compared to a 3C criterion than the present study (MD: 2.4 %) [6]. Body fat percentage results in the present study demonstrated better agreement between ADP and a 4C criterion for African American/Black participants (SEE = 1.6 %, MD = 1.8%) compared to previous literature in a younger male populations (SEE = 4.7%; MD = -3.6 %). In Asian participants, ADP has primarily been validated against a DXA criterion, which makes comparison difficult, however, SEE (2.6 % vs. 1.6%) [113] and MD (-3.9 % vs. 2.1 %) [13] values were larger than the current study.

Several studies have investigated the validity of BIA devices [3,15,21,35,74], however, few have utilized a multi-compartment criterion for comparison [15,26,129]. A study by Bosy-Westphal et al.[15], similar to the present study investigated the validity of FFM measures in Hispanic, Caucasian, African American and Asian participants and reported MD values of 0.4 kg, 0.7 kg, 1.5 kg, 0.7 kg, respectively, and TE values of 1.9 kg, 2.1 kg, 2.2 kg, and 1.9 kg, respectively. These results align with the IB TE and MD values reported in the present study, but are smaller than BIS error observed in this study. In a larger sample of Asian individuals (n=298), SEE (4.5%) was larger than both bioimpedance devices in the present study (2.7 – 3.1 %). An older study evaluating BIA FFM estimates compared to a 4C model in Hispanic females reported variable results depending on the specific regression equation utilized [129]; overall results demonstrated lower SEE (1.3 - 2.0 kg) compared to BIS and similar to IB and similar TE (1.6 – 3.2 kg) compared to the current study, with the exception of the Van Loan [68] equation which performed poorly for FFM estimates (TE = 4.6 kg). To our knowledge, only one study has evaluated the validity of the IB device compared to a 4C criterion, although the 4C equation utilized bioelectrical impedance technology as opposed to isotope dilution. This study evaluated 146 African American, Caucasian and Hispanic individuals and reported larger TE (5.1 – 5.5 %) and SEE (4.8 – 5.2 %) values than the current study for the total sample [45]. Within each race/ethnicity, MD results were similar for African American/Black individuals (-0.34 %) and larger for Caucasian/White (-2.13 %) and Hispanic (1.4 %) participants compared to our study findings. Results from the current study suggest the BIS, which is commonly used in clinical settings [60,64,114], should be evaluated in larger, multi-ethnic populations to ensure validity.

Bioelectrical impedances devices, which demonstrated the largest TE and SEE for all races/ethnicities, require several assumptions regarding body proportion, body density and resistivity of tissue [36]. A previous investigation in our laboratory (Blue et al. – unpublished) observed significantly different relative arm and leg lengths between races/ethnicities; African American/Black participants had longer arms and legs compared to Asian and Caucasian/White individuals. Device estimates may be improved by allowing users to incorporate a measure of limb length or utilizing a different body proportion coefficient. Additional components that may influence BIA/BIS measures such as fat distribution, fat-free body density, total body density and TBW:FFM ratio should be investigated further to determine if adjusting standard coefficients and algorithms may improve accuracy when evaluating a broad, diverse sample.

The current study results suggests the multi-compartment models evaluated can be utilized in a multi-ethnic sample, as well as in each individual race/ethnicity to obtain highly valid results for both %fat and FFM. As TBW is a large component of multi-compartment models, utilizing isotope dilution provided the most valid estimates (D_2O 3C) even beyond models that measured an additional body constituent (i.e. Mo in BIS 4C model). However, as isotope dilution is a time-consuming and expensive method, the use of either BIS or IB in multi-compartment models for measures of TBW will still provide accurate estimates of body composition. Further research needs to evaluate the use of the DXA-BV 4C in a multi-ethnic sample before including it as criterion to validate additional devices. Mean estimates from 4C BIS and 3C BIS may underestimate %fat (1-2%) and overestimate FFM (~1 kg), respectively; while DXA-BV 4C may overestimate %fat (1-4%). Additionally, our study results suggest, the single device estimates from DXA and ADP are valid for mean estimates, although individual variability may be high. IB, BIS, and DXA-BV 4C measures demonstrated the greatest error compared to all other models; BIS estimates were not valid in African American/Black, Caucasian/White and Multi-racial samples. To our knowledge, this is one of the first studies to include individuals who identify as more than one race/ethnicity. Results for this cohort were similar to the other all other races/ethnicities, potentially minimizing concern that error would increase when physiological differences were observed within a racial cohort (i.e. different limb length for an individual who identifies as Hispanic and Caucasian compared to African American and Asian). Investigators evaluating primarily Asian or Hispanic subjects should be particularly cautious regarding DXA-BV 4C and BIS measures. Furthermore investigators should be aware of the potential for each device to over or underestimate %fat and FFM depending on individual variability. Overall, the most accurate estimates for all races/ethnicities were obtained from D_2O 3C, BIS 4C, BIS 3C (TE = 0.9 – 2.4 %), followed by DXA, ADP, IB (TE= 2.5 – 2.9%), and then DXA-BV 4C and BIS (TE = 3.79 – 4.12%). Results did not vary significantly between races/ethnicities, except for DXA-BV 4C (less accurate for Asian and Hispanic) and BIS (less accurate for African American/Black, Caucasian/White, Multi-racial) measures.

CHAPTER V: MANUSCRIPT 2

Validity of total body water estimates from two multi-frequency bioelectrical impedance devices in multiple races and ethnicities

Introduction

Body composition is an important component of health and is frequently evaluated to predict obesity-related disease risk and health outcomes [11,86]. Multi-compartment models are the criterion for measuring body composition as they assess the various constituents of the body including fat mass (FM), fat-free mass (FFM), lean soft tissue, total body water (TBW) and bone mineral content (BMC) [49,148]. Total body water is a key body compartment, is the largest component of FFM (approximately 73% of FFM is composed of water), and therefore may induce the largest amount of variability in body composition assessments [150]. Previous literature has observed that multi-compartment body composition models that include a measure of TBW are the most accurate and reliable measures [148]. Additionally, TBW is an important measure for clinical populations; as an assessment of body fat percentage (%fat), and in individuals at risk for lymphedema or shifts in body fluid such as individuals with cancer [64], heart failure [114] and chronic hemodialysis patients [60].

Isotope dilution techniques are the criterion for measuring TBW. Specifically, deuterium dilution analysis via mass spectroscopy is predominately used to evaluate TBW. However, this technique is expensive and time-intensive; thus, several bioelectrical impedance devices have been developed to estimate TBW. For bioimpedance measures, the human body and limbs are assumed to be a cylindrical shape, and therefore the equation to determine TBW volume is: $V = \rho L^2/R$ where ρ is the resistivity of tissue coefficient, L is the height of the subject and R is the measured resistance [36]. Bioelectrical impedance analysis (BIA) devices use a single frequency (i.e. Omron HBF-306C, Beurer BF22, RJL Systems) or multiple frequencies (i.e. Seca mBCA, Tanita MC-780U) in combination with algorithms based on population characteristics to estimate TBW and %fat. Bioelectrical impedance spectroscopy (BIS) devices utilize a range of multiple frequencies (1-1000 kHz) and Cole-Cole plot analysis to measure TBW. Although BIS does not rely on population characteristic assumptions to determine TBW, there are still several assumptions including body proportion, body density, and resistivity of tissue. Previous literature has observed differences in these compartments for individuals of different races/ethnicities. African American/Black individuals have been observed to have longer legs compared to Caucasian/White individuals [30,139], and Asian individuals have been reported to have a shorter legs than Caucasian/White participants [30]. Additionally, differences in compartments of fat free body density (i.e. bone mineral content/fat free mass ratio) have been observed between races/ethnicities [27,138,139] which may lead to differences in total body density.

Although bioelectrical impedance measures of TBW have been validated against the isotope dilution criterion in general populations [9,85,96], participants have primarily been Caucasian/White, or race was not reported. To our knowledge, in minority populations, the validity of TBW has only been evaluated with BIA devices [15,48], relying on proprietary algorithms incorporating population characteristics; no studies have investigated the validity of TBW measures by BIS devices in minority populations. As TBW is a key component for both multi-compartment criterion body composition models and as a stand-alone affordable and potentially portable technique to estimate body composition, it is imperative to accurately estimate TBW via bioelectrical impedance. Therefore, the purpose of the current study was to assess the validity of two multi-frequency bioelectrical impedance devices for measures of total body water compared to a deuterium dilution criterion in a multi-ethnic sample stratified by race/ethnicity. Secondary analyses evaluated characteristics (body density, TBW:FFM Ratio, limb length, Waist:Hip ratio) of each race/ethnicity that may contribute to validity of TBW measures.

Materials and Methods

Participants

One hundred and nine adults (55% Female, Table 1) enrolled in the present study. A detailed description of participant enrollment and demographics are previously described (Blue et al. -unpublished). Participants were stratified by race/ethnicity in the following cohorts: African American/Black (n=10 male; n=12 female), Asian (n=10 male; n=12 female), Caucasian/White (n=10 male; n=12 female), Hispanic/Latinx (n=10 male; n=12 female), and Multi-Racial (n=9 male; n=12 female; incl. Hispanic and White [n=11], Hispanic and Black [n=1], Black and White [n=6], Black and Asian [n=3]). Participants were excluded if their body mass index (BMI) was <18.5 or >39.99 kg/m^2, if they were pregnant or planning to become pregnant, if they had gained or lost greater than 3 kg in previous two months, had been diagnosed with a musculoskeletal disease; had been diagnosed with a musculoskeletal injury limiting daily activities in the previous 3 months, actively participated in 7 days per week of resistance or aerobic training, had used anabolic steroids in previous year, had a self-identified or clinically diagnosed eating disorder, or had been diagnosed with a disease that may have resulted in significant changes in total body water (i.e. renal disease) or weight status (i.e. thyroid abnormalities). Prior to enrollment, all participants signed a consent form approved by the University's Biomedical Review Board for the protection of human subjects.

Experimental Design

Participants reported to the laboratory for a single testing session after a 12 hour fast including abstention from caffeine, alcohol and tobacco. Participants refrained from strenuous exercise for a minimum of 24 hours prior to testing. Following enrollment, participants provided a urine sample to assess hydration by urine specific gravity (USG = 1.002-1.029) and a saliva sample for baseline total body water (TBW) estimates. A research technician measured height to the 0.1 cm using a portable stadiometer (Perspective Enterprises, Portage, MI, USA) and weight to the nearest 0.1 kg using a calibrated scale (Tanita Inc., Tokyo, Japan). Bioelectrical impedance device measurements were completed followed by ingestion of deuterium oxide (D_2O) for TBW measurement. After a three-hour equilibration period, participants provided a second saliva sample for analysis of TBW. Anthropometric (limb length, waist and hip circumference) and additional body composition assessments were completed during the equilibration period. Detailed descriptions of the additional body composition assessments are previously described (Blue et al. -unpublished).

Deuterium Dilution

Total body water was measured by a criterion three-hour D_2O dilution protocol in accordance with the International Atomic Energy Agency guidelines for isotope dilution. A research technician prepared a large batch of the D_2O solution composed of 5 L of tap water and 515 g of D_2O. Individual doses of 58 g, 67 g, 79 g, 95 g and 114 g were prepared to ensure an excess of 0.05 g ^2H per kilogram of body mass [10,88]. Based on body mass and sex, participants consumed one of the pre-measured doses of the D_2O solution after providing a 2 mL baseline saliva sample. The exact dose of D_2O was recorded to the nearest 0.01 g for each participant. During the 3-hour equilibration period, participants were allowed to have up to 250 mL of water. The volume consumed by each participant was recorded. Three hours following ingestion, participants provided a post saliva sample. Saliva samples were stored in -20°C freezer until batch analysis was completed in triplicate via isotope-ratio mass spectroscopy at the University of Wisconsin Isotope Ratio Mass Spectrometry Laboratory. The baseline and D_2O-enriched saliva samples were used to calculate TBW including the correction factor for nonaqueous exchange of D_2O [115].

Bioelectrical Impedance

Two multi-frequency bioelectrical impedance devices were used to assess TBW. For the first bioelectrical impedance device (BIS: SFB7 ImpediMed, Queensland, Australia [10 - 500 kHz]) participants laid supine on a table with arms separated from the torso and legs separated. Prior to testing, height, weight, age, and sex were entered into the device, and each electrode site was cleaned with an alcohol wipe. Two electrodes were placed 5 cm apart on the dorsal side of the right wrist and hand, and two electrodes were placed on the dorsal side of the right ankle and foot for total body measures. Device default settings for resistivity coefficients, body density (1.05 kg/L) and the body proportion constant (4.30 a.u.) were used to estimate TBW.

For the second bioelectrical impedance device (IB: InBody 770; Biospace Co., Seoul, Korea [1-1000 kHz]), participants were asked to stand upright for 5 minutes prior to measurement of TBW. Participants stood barefoot on the device with their soles in contact with the foot electrodes with legs separated and were instructed to grasp the device handles with their palm, fingers and thumb making contact with the hand electrodes. Arms were raised to separate from the torso and participants were asked to stand still during measurement. Device software automatically estimated TBW in L.

Anthropometrics

Waist and hip circumferences were measured by placing a measuring tape with a Gulick attachment around the trunk at the top of the iliac crest and the widest portion of the buttocks, respectively. Participants stood with feet together and body weight evenly distributed on both feet. Limb length measures were taken on the right side of the body. Participants laid supine with the right leg and arm fully extended. With the arm in a pronated position, a measuring tape was used to measure the arm length from the acromion process to the radial styloid process. Leg length was measured from the greater trochanter to the lateral malleolus. Measurements were recorded to the nearest 0.1 cm.

Statistical Analysis

Mean, standard deviation, mean difference and confidence intervals were calculated for each device for the entire sample and within each racial/ethnic cohort. Total error (TE $=\sqrt{\sum[\text{predicted-actual}]^2/n}$), standard error of the estimate (SEE $=\sqrt{\sum[\text{predicted-actual}]}\bullet\sqrt{1-r^2}$) and Pearson's correlation coefficients were completed to determine the agreement of each bioelectrical impedance device for estimates of TBW compared to the D_2O criterion. TBW accounts for approximately 73% of total body FFM (TBW:FFM range: 66.8 – 75.2%), therefore SEE/TE subjective rating values (According to Heyward and Wagner [49]) were adjusted to match 73% of TE/SEE expected from the total body. For excellent to fair agreement, SEE/TE values should be between 1.32 – 3.29 L. Paired-samples t-tests were also performed to evaluate the agreement between body composition models. To assess individual variability, Bland-Altman plots for TBW were constructed and proportional bias was assessed by linear regression analyses for each device. For the secondary aim, separate one-way analysis of variance (ANOVA) tests were conducted to evaluate differences in body composition characteristics (body density, limb length, Waist to hip ratio and the ratio of TBW from D_2O to FFM from 4C) of each race/ethnicity; Tukey HSD post-hoc analyses were performed to assess the differences between groups. Pearson's correlation coefficients assessed the relationship between the difference in bioelectrical impedance device and D_2O TBW estimates to body composition characteristics. An alpha level of 0.05 was set a priori. Statistical analyses were performed using SPSS (IBM Corp, IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY).

Results

For the full multi-ethnic sample, BIS estimates demonstrated good to fairly good agreement (TE=2.55 L, SEE=2.00 L, R^2=0.951) and IB estimates were excellent to very good (TE=1.95 L, SEE=1.43 L, R^2=0.975) compared to the criterion TBW measure. However, significant overestimation of mean differences ($p<0.001$) were observed for BIS (Mean ±SD: 40.1 ± 9.0 L) and IB (39.8 ± 9.0 L) compared to D_2O (38.5 ± 9.0 L).

BIS estimates for Asian, African American, Hispanic were good to fairly good, and fairly good to fair for Caucasian/White and Multi-racial participants. For the IB, estimates were excellent to very good for all races/ethnicities, except Caucasian/White which demonstrated good to fairly good results. For BIS measures, using Caucasian/White (TE: 2.99 L) as a comparator, TBW error was lower for all other races/ethnicities (TE: 2.30 – 2.66 L) (Table 8). For the IB, Caucasian/White (TE =2.40 L) estimates were less valid compared to all other races/ethnicities (TE: 1.64 – 2.01 L).

BIS estimates were significantly different from the D_2O measures for Asian, Caucasian/White, Hispanic and Multi-racial individuals ($p<0.05$) (Table 9), with both devices overestimating TBW by approximately 1-2 L. For African American/Black participants, the IB was significantly different from D_2O (p=0.015), but there was no difference for BIS and D_2O measures (p=0.168). For all races/ethnicities, based on the lower SEE and TE, the IB produced more valid estimates compared to BIS estimates.

Bland-Altman plot and regression analyses (Figure 7) demonstrated individual variability for the total sample was greater for the BIS compared to IB. The 95% limits of agreement were more narrow for IB (-1.49 – 4.13 L) compared to BIS (-2.36 – 5.51 L). Proportional bias was not present for either device (p= 0.991 – 0.999).

Secondary analyses demonstrated there were no significant differences between races/ethnicities for body density (1.032 – 1.063 g/cm^3; p=0.410), TBW:FFM ratio (0.708 – 0.713; p=0.766), or Waist:Hip ratio (0.77 – 0.79; p=0.836). There was a significant difference in relative arm length (Arm length (cm)/Height (cm); p<0.001) and relative leg length (Leg length (cm)/Height (cm); p<0.001). African American/Black participants had significantly longer arm length relative to height (0.334) compared to Asian (0.322), Caucasian/White (0.322) and Hispanic (0.321) participants; African American/Black (AA) and Multi-racial (MR) participants had significantly longer leg length relative to height (AA: 0.500; MR: 0.496) compared to Asian (0.478) and Caucasian/White (0.477). In the full sample, the difference between BIS and D_2O estimates was significantly correlated to relative arm (r=-0.313, p=0.001) and leg length (r=-0.295, p=0.002); the difference between IB and D_2O was significantly correlated to relative arm length (r=-0.205, p=0.033).

Discussion

For TBW measures in the full multi-ethnic sample and within each race/ethnicity, validity statistics suggested bioelectrical impedance device estimates were valid. However, results varied slightly by device and race/ethnicity. All races/ethnicities had significantly different ($p<0.05$) mean estimates for TBW between D_2O and BIS and IB, except African American/Black BIS measures (D_2O: 42.25 L vs BIS: 42.94 L). However, validity statistics indicated both bioelectrical impedance devices provided acceptable estimates. The BIS produced more valid results in Asian, African American/Black and Hispanic individuals compared to Caucasian/White and Multi-racial samples; all measures were considered valid ranging from good to fair. The IB results were more valid for TBW compared to BIS, with estimates ranging from excellent to very good for all races, with the exception of Caucasian/White which was good to fairly good. Bland-Altman plot analyses revealed similar results for individual variability with the IB demonstrating narrower limits of agreement compared to BIS. Body composition characteristics were similar across all races/ethnicities, however, there were differences in relative arm and leg length, which were found to be significantly related to the difference between the bioelectrical impedance devices and D_2O TBW measures. Both arm and leg length were negatively correlated (Arm: $r=-0.313$; Leg: $r=-0.295$) to the difference in BIS and D_2O estimates and arm length was negatively correlated ($r=-0.205$) to the difference in IB and D_2O measures.

Previous literature assessing the validity of TBW measures in a multi-ethnic sample, stratified by race/ethnicity is limited. However, our results are similar to previous literature evaluating different bioelectrical impedance analysis devices. A previous study [15] validating a different multi-frequency BIA device (seca mBCA) demonstrated smaller TE compared to the BIS device and similar results compared to the IB in the present study (TE=1.3 – 1.7 L) in a sample of Caucasian, Asian, African American and Hispanic adults. Similar to the present study, the largest TE was reported for the Caucasian sample (TE=1.7 L). However, the seca mBCA device demonstrated smaller mean differences compared to the devices in the present study (-0.3 – 0.4 L vs. 0.7 – 2.0 L). Another study evaluating the validity of a multi-frequency BIA device (Humanlm, Dietosystems) in a large sample of Asian individuals (n=318) in Indonesia, reported similar SEE values (1.2 – 1.6 L) as the IB in the present study (SEE=1.2-1.7 L)[48]. The TBW measures were estimated using a population specific regression equation; this study also compared TBW results from the isotope dilution criterion to estimates utilizing previous bioimpedance equations created in Caucasian populations (i.e. Lukaski [73]) and found larger mean differences (0.6 – 4.9 L). This supports the use of devices that limit the need for population based information such as the IB and BIS evaluated in the present study.

Valid estimates of TBW reported in the current study are similar to previous studies that do not include multi-ethnic samples. A study in 28 Caucasian males and females evaluating the same BIS device in the present study reported similar SEE (2.12 L) and TE (2.21 L) values, with males reporting larger error values (SEE = 2.7 L; TE = 2.73 L)[85]. The present study consisted of 55% female participants, thus future studies evaluating the influence of sex differences on TBW estimates may be warranted. Two smaller studies (n=13-14), assessed the validity of a Xitron 4000B device, utilizing similar technology to the BIS, and reported smaller TE, but similar SEE results to the BIS in the present study (TE: 1.6 L; SEE: 2.2 L)[9,96].

Both BIA and BIS devices include assumptions regarding body proportion, body density and resistivity of tissue [36]. Therefore, the current study evaluated racial/ethnic differences of various body composition characteristics; there were no significant differences between races/ethnicities for body density, waist:hip ratio or TBW:FFM ratio. The lack of differences supports the similar TE and SEE results across various races/ethnicities reported in the present study. However, there were differences in relative arm and leg length, and both were related to the difference observed between the criterion and BIS TBW estimates. The negative correlation indicates that the difference in BIS and D_2O estimates increased as arm length and leg length decreased. Asian and Caucasian/White individuals in the present study were found to have shorter leg and arm lengths than African American/Black participants, therefore, it supports the larger mean differences observed for Asians (1.99 L) and Caucasians (2.00 L), compared to African American/Black participants (0.68 L). This suggests the body proportion coefficient utilized by the BIS was established in individuals with longer limbs, improving accuracy in populations with similar body proportion. Similarly, for the IB, as arm length increased, the MD between IB and D_2O estimates decreased. However, leg length was not significantly correlated to the difference between measures. As legs are a larger portion of our overall body proportion, the non-significant relationship observed may support the smaller MD values for the IB in comparison to the BIS. Although the equation and exact procedure (i.e. regression analysis vs. Cole plot analysis) used to measure TBW by IB is proprietary, our results suggests a different body proportion or limb length to height ratio is used for the IB compared to BIS. Limb length differences are commonly observed between races/ethnicities [30,139]; bioelectrical impedance devices that allow users to input limb length or utilize a more robust body proportion coefficient will likely produce better results in a broad multi-ethnic sample, as observed with the IB in the present study.

Results from the current study suggest the two multi-frequency bioelectrical impedance devices in the present study can produce valid TBW estimates compared to the D_2O criterion in a multi-ethnic sample and within each race/ethnicity. TBW estimates from the IB may be more accurate compared to the BIS. Although both device estimates were considered valid via TE and SEE values, these devices still may overestimate TBW by 1-2 L. The influence this overestimation may have on multi-compartment models that utilize bioelectrical impedance instead of D_2O for TBW measures should be evaluated in future studies. The present study included a broad range of individuals within each racial/ethnic category (age: 18-45 yrs, %fat: 6-48%), which increased variability, potentially limiting our ability to observe body characteristic (i.e. body density, TBW:FFM, Waist:Hip) differences with the current sample size. However, the finding that relative arm and leg length was related to the difference observed between methods suggests device body proportion coefficients may need to be adjusted when evaluating a broad, multi-ethnic sample. Bioelectrical impedance devices are frequently used to measure TBW for multi-compartment models, and as time-efficient portable body composition assessments; therefore, it is necessary to ensure TBW estimates are accurate across multiple populations. Based on the current study results, when utilizing TBW in multi-compartment models or to assess body composition, investigators and clinicians should consider using the IB in lieu of the BIS in broad, multi-ethnic populations.

5.1 Tables: Aim 1 and Aim 2

Table 1. Participant characteristics. Presented as mean ± SD

	N	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	%Fat	FFM (kg)
Total	110	26.5 ± 6.9	169.4 ± 9.4	72.8 ± 14.4	25.3 ± 4.0	25.7 ± 9.5	54.1 ± 12.7
Asian	22	26.1 ± 7.0	167.3 ± 8.0	64.5 ± 10.4	23.0 ± 3.2	25.1 ± 7.9	49.2 ± 10.1
African American/Black	22	26.1 ± 4.4	170.5 ± 9.0	80.9 ± 16.0	27.7 ± 4.4	26.4 ± 9.5	59.3 ± 13.7
Caucasian/White	22	29.3 ± 8.3	172.3 ± 9.5	73.9 ± 11.8	24.9 ± 3.4	24.5 ± 10.6	55.8 ± 12.2
Hispanic	22	24.4 ± 5.8	165.0 ± 9.6	68.8 ± 14.7	25.1 ± 3.7	27.2 ± 8.4	50.1 ± 12.4
Multi-Racial	21	27.0 ± 7.8	172.2 ± 9.6	76.2 ± 14.2	25.7 ± 4.3	25.1 ± 11.7	56.8 ± 12.8

BMI = body mass index; %Fat = body fat percentage; FFM = fat free mass *%fat and FFM

Table 2. Distribution of sample by body mass index and age

BMI (kg/m^2)	**< 25**			**25 to 29.9**			**≥30**		
Age (y)	**18-26**	**27-35**	**36-45**	**18-26**	**27-35**	**36-45**	**18-26**	**27-35**	**36-45**
Non-Hispanic White	6 (27.2)	3 (13.6)	3 (13.6)	4 (18.2)	3 (13.6)	1 (4.5)	0	0	2 (9.1)
Non-Hispanic Black	3 (13.6)	3 (13.6)	0	6 (27.2)	2 (9.1)	1 (4.5)	4 (18.2)	3 (13.6)	0
Hispanic	9 (40.9)	2 (9.1)	1(4.5)	6 (27.2)	2 (9.1)	0	1 (4.5)	1 (4.5)	0
Asian	12 (54.5)	3 (13.6)	3 (13.6)	2 (9.1)	0	0	0	0	1 (4.5)
Multi-Racial	7 (33.3)	1 (4.8)	3 (14.3)	4 (19.0)	1 (4.8)	1 (4.8)	2 (9.5)	1 (4.8)	1 (4.8)

Frequency (% of race/ethnic sample)

Table 3. Methods for 4-compartment (4C) model body composition estimates

	BV	**TBW**	**Mo**	**BM**
Criterion 4C	ADP	D_2O	DXA	Calibrated electronic scale
BIS 4C	ADP	BIS	DXA	Calibrated electronic scale
DXA-BV 4C	$* \mathrm{BV\ (L)} = \frac{\mathrm{FM}}{0.84} + \frac{\mathrm{LM}}{1.03} + \frac{\mathrm{BMC}}{11.63} - 3.12$	BIS	DXA	Calibrated electronic scale

BV = body volume; TBW = total body water; Mo = total body bone mineral; BM=body mass; ADP= air displacement plethysmography; D_2O = deuterium dilution; DXA = dual-energy X-ray absorptiometry; BIS=bioelectrical impedance spectroscopy; FM = fat mass; LM = lean mass; *equation described by Smith-Ryan et al. (2017) using DXA-derived FM, LM, BMC.

Table 4. Methods for 3-compartment (3C) model body composition estimates

	BV	**TBW**	**BM**
D_2O 3C	ADP	D_2O	Calibrated electronic scale
BIS 3C	ADP	BIS	Calibrated electronic scale

BV = body volume; TBW = total body water; BM=body mass; D_2O = deuterium dilution; ADP= air displacement plethysmography; BIS=bioelectrical impedance spectroscopy

Table 5. Validity statistics comparing the 4C criterion with four multi-compartment models for measures of body fat percentage and fat-free mass

		%Fat					FFM (kg)				
		Mean (SD)	**TE**	**SEE**	**R^2**	**Rating**	**Mean (SD)**	**TE**	**SEE**	**R^2**	**Rating**
Asian	**Criterion**	24.25 (6.58)					49.23 (10.07)				
	BIS 4C	**22.19 (6.76)**	2.37	1.35	0.958	Excellent-Ideal	**50.62 (10.64)**	1.69	0.79	0.994	Ideal
	DXABV 4C	**20.78 (6.19)**	4.20	2.41	0.865	Fair–Fairly good	**51.29 (9.14)**	2.41	0.92	0.992	Very good-Excellent
	D_2O 3C	**25.16 (6.26)**	1.02	0.37	0.997	Ideal	**48.62 (9.77)**	0.70	0.21	0.999	Ideal
	BIS 3C	**22.86 (6.46)**	2.03	1.51	0.947	Excellent-Ideal	**50.17 (10.41)**	1.35	0.92	0.992	Ideal
African American/ Black	**Criterion**	26.42 (9.48)					59.35 (13.72)				
	BIS 4C	25.87 (7.97)	1.99	1.38	0.979	Excellent-Ideal	59.82 (13.35)	1.61	1.54	0.987	Ideal
	DXABV 4C	26.96 (8.11)	3.39	3.34	0.876	Good–Very good	58.62 (11.61)	2.61	1.59	0.987	Very good-Excellent
	D_2O 3C	**27.18 (9.28)**	0.87	0.40	0.998	Ideal	**58.73 (13.52)**	0.72	0.33	0.999	Ideal
	BIS 3C	26.57 (7.62)	2.35	1.69	0.968	Excellent-Ideal	59.26 (13.12)	1.84	1.82	0.982	Ideal
Caucasian /White	**Criterion**	24.49 (10.57)					55.78 (12.19)				
	BIS 4C	**22.66 (9.57)**	2.73	1.90	0.968	Very good-Excellent	**57.17 (12.09)**	2.09	1.59	0.983	Excellent-Ideal
	DXABV 4C	23.04 (9.07)	3.51	3.11	0.914	Good–Very good	56.64 (10.37)	2.47	1.63	0.982	Very good-Excellent
	D_2O 3C	**25.36 (10.25)**	0.98	0.23	0.999	Ideal	**55.13 (11.94)**	0.74	0.27	0.999	Ideal
	BIS 3C	**23.31 (9.15)**	2.68	2.15	0.959	Very good-Excellent	**56.69 (11.84)**	2.04	1.86	0.977	Excellent-Ideal
Hispanic	**Criterion**	27.20 (8.38)					50.13 (12.37)				
	BIS 4C	**25.39 (8.11)**	2.35	1.53	0.967	Excellent-Ideal	**51.40 (12.59)**	1.67	1.07	0.993	Ideal
	DXABV 4C	**25.05 (7.79)**	4.31	3.81	0.794	Fair–Fairly good	**51.21 (10.50)**	2.55	1.56	0.984	Very good-Excellent
	D_2O 3C	**28.03 (7.98)**	0.97	0.33	0.999	Ideal	**49.53 (11.99)**	0.74	0.21	0.999	Ideal
	BIS 3C	**26.01 (7.71)**	2.17	1.80	0.954	Excellent-Ideal	**50.95 (12.24)**	1.51	1.30	0.989	Ideal
Multi-Racial	**Criterion**	25.12 (11.74)					56.78 (12.78)				
	BIS 4C	**23.85 (10.59)**	2.29	1.66	0.980	Excellent-Ideal	**57.80 (12.52)**	1.86	1.59	0.985	Excellent-Ideal
	DXABV 4C	24.27 (10.64)	3.51	3.43	0.914	Good–Very good	57.16 (10.86)	2.46	1.73	0.982	Very good-Excellent
	D_2O 3C	**25.86 (11.49)**	0.88	0.43	0.999	Ideal	**56.21 (12.54)**	0.70	0.35	0.999	Ideal
	BIS 3C	24.45 (10.20)	2.35	1.84	0.976	Excellent-Ideal	57.36 (12.28)	1.89	1.81	0.980	Excellent-Ideal

%fat = body fat percentage; FFM = fat-free mass; SD = standard deviation; TE = total error; SEE = standard error of the estimate; 4C = four-compartment model; BIS = bioelectrical impedance spectroscopy (Impedimed); DXA = dual-energy X-ray absorptiometry; BV = body volume; D2O = deuterium dilution; 3C= three-compartment model; bold indicates significant difference from criterion ($p<0.05$); subjective rating scale according to Heyward and Wagner [50] adapted from Lohman [69]

Table 6. Validity statistics comparing the 4C criterion with four single device models for measures of body fat percentage and fat-free mass

		% Fat					**FFM (kg)**				
		Mean (SD)	**TE**	**SEE**	**R^2**	**Rating**	**Mean(SD)**	**TE**	**SEE**	**R^2**	**Rating**
Asian	DXA	**26.62 (6.23)**	2.61	1.10	0.972	Very good-Excellent	**47.86 (9.70)**	1.59	0.76	0.994	Ideal
	ADP	**22.20 (6.61)**	2.59	1.61	0.940	Very good-Excellent	**50.45 (10.07)**	1.58	0.95	0.991	Ideal
	BIS	**22.81 (6.78)**	3.13	2.73	0.828	Good–Very good	**50.33 (11.14)**	2.20	1.55	0.976	Excellent
	IB	23.70 (6.28)	3.14	3.13	0.773	Good–Very good	49.59 (9.89)	2.09	2.11	0.956	Excellent – Ideal
African American/ Black	DXA	**28.61 (8.94)**	2.58	1.32	0.981	Very good-Excellent	**57.88 (13.61)**	1.83	1.10	0.994	Excellent - Ideal
	ADP	**24.62 (9.52)**	2.41	1.62	0.971	Excellent-Ideal	**60.67 (13.30)**	1.76	1.13	0.993	Ideal
	BIS	27.61 (6.72)	4.63	4.24	0.799	Poor–Fairly good	58.66 (13.33)	3.44	3.45	0.937	Fairly good – Good
	IB	26.90 (9.70)	3.21	3.22	0.764	Good–Very good	59.05 (13.82)	2.11	2.12	0.976	Excellent – Ideal
Caucasian/ White	DXA	**26.11 (9.32)**	2.65	1.85	0.969	Very good-Excellent	**54.91 (11.42)**	1.76	1.41	0.987	Ideal
	ADP	**23.01 (11.29)**	2.27	1.54	0.979	Excellent-Ideal	**56.84 (12.49)**	1.62	1.20	0.990	Ideal
	BIS	23.12 (7.97)	4.86	4.50	0.819	Poor–Fairly good	56.99 (11.74)	3.74	3.62	0.912	Fairly good – Good
	IB	23.21 (9.82)	3.43	3.25	0.906	Good–Very good	56.70 (11.33)	2.52	2.31	0.964	Good – Very good
Hispanic	DXA	**29.61 (7.42)**	2.80	1.15	0.981	Very good-Excellent	**48.67 (11.73)**	1.78	0.85	0.995	Ideal
	ADP	**25.22 (8.38)**	2.60	1.59	0.964	Very good-Excellent	**51.38 (12.32)**	1.71	1.20	0.991	Ideal
	BIS	26.18 (7.24)	3.49	3.39	0.836	Good–Very good	51.01 (12.46)	2.66	2.55	0.958	Good – Very good
	IB	26.77 (8.29)	2.44	2.44	0.915	Very good-Excellent	50.51 (12.20)	1.81	1.81	0.979	Ideal
Multi-Racial	DXA	**27.14 (10.52)**	2.91	1.86	0.975	Very good-Excellent	**55.53 (12.09)**	2.05	1.55	0.985	Excellent - Ideal
	ADP	**22.96 (12.68)**	2.71	1.32	0.987	Very good-Excellent	**58.43 (13.61)**	2.12	1.03	0.993	Excellent - Ideal
	BIS	25.18 (8.57)	4.25	3.46	0.913	Poor–Fairly good	56.78 (11.57)	3.39	3.38	0.930	Fairly good – Good
	IB	25.12 (11.31)	2.74	2.80	0.943	Very good-Excellent	56.87 (12.65)	2.20	2.12	0.969	Excellent

%fat = body fat percentage; FFM = fat-free mass; SD = standard deviation; TE = total error; SEE = standard error of the estimate; DXA = dual-energy X-ray absorptiometry; ADP = air displacement plethysmography; BIS = bioelectrical impedance spectroscopy (Impedimed); IB = Inbody; bold indicates significant difference from criterion ($p<0.05$); subjective rating scale according to Heyward and Wagner [50] adapted from Lohman

Table 7. Comparison of body volume measured via air displacement plethysmography and body volume derived from dual-energy X-ray absorptiometry

	Asian	African American/Black	Caucasian/White	Hispanic	Multi-Racial
DXA BV (L)	61.53 (10.49)	78.02 (16.33)	70.80 (12.08)	66.15 (15.02)	73.14 (14.73)
ADP BV (L)	61.78 (9.94)	77.58 (15.69)	70.60 (11.50)	66.09 (14.29)	72.91 (14.10)
MD [CI] (L)	-0.24 [-0.52, 0.03]	**0.44** [0.10, 0.78]	0.19 [-0.14, 0.52]	0.07 [-0.31, 0.45]	0.23 [-0.13, 0.59]
TE (L)	0.65	0.87	0.75	0.83	0.81
SEE (L)	0.27	0.42	0.45	0.43	0.47
R^2	0.999	0.999	0.998	0.999	0.999

BV = body volume; DXA = dual-energy X-ray absorptiometry; ADP = air displacement plethysmography; MD = mean difference; CI = 95% confidence interval of difference; TE = total error; SEE = standard error of the estimate; bold indicates significant difference between methods ($p<0.05$)

Table 8. Validity statistics comparing bioelectrical impedance devices and deuterium dilution total body water estimates

	BIS				IB			
	TE (L)	**SEE (L)**	R^2	**Rating**	**TE (L)**	**SEE (L)**	R^2	**Rating**
Asian	2.42	1.07	0.978	Fairly good - Good	2.01	1.45	0.960	Very good - Excellent
African American/Black	2.30	2.24	0.951	Fairly good - Good	1.64	1.45	0.979	Very good - Excellent
Caucasian/White	2.99	2.25	0.931	Fair – Fairly good	2.40	1.68	0.961	Fairly good – Good
Hispanic/Latinx	2.38	1.49	0.971	Fairly good - Good	1.82	1.22	0.981	Very good - Excellent
Multi-Racial	2.66	2.28	0.932	Fair – Fairly good	1.80	1.18	0.982	Very good - Excellent

BIS = Impedimed SFB7 bioelectrical impedance spectroscopy; IB = Inbody 770 bioelectrical impedance device; TE = total error; SEE = standard error of the estimate

Table 9. Mean (SD) and mean difference [95% confidence interval] between bioelectrical impedance devices and deuterium dilution TBW estimates

	Asian	**African American/Black**	**Caucasian/White**	**Hispanic**	**Multi-Racial**
TBW (L)					
D_2O	34.85 (7.31)	42.25 (10.12)	39.72 (8.55)	35.52 (8.72)	40.24 (8.70)
BIS	36.84 (8.15)	42.94 (9.76)	41.72 (8.59)	37.34 (9.12)	41.71 (8.47)
IB	36.27 (7.25)	43.08 (10.02)	41.47 (8.27)	36.88 (8.87)	41.51 (9.19)
MD_1 [CI]	**1.99** [1.36, 2.62]	0.68 [-0.31, 1.68]	**2.00** [0.99, 3.01]	**1.81** [1.11, 2.51]	**1.46** [0.43, 2.50]
MD_2 [CI]	**1.41** [0.77, 2.06]	**0.82** [0.18, 1.47]	**1.75** [1.00, 2.49]	**1.36** [0.81, 1.91]	**1.27** [0.67, 1.86]

TBW = total body water; D_2O = deuterium dilution; BIS = Impedimed SFB7 bioelectrical impedance spectroscopy; IB = Inbody 770 bioelectrical impedance device; MD = mean difference; CI =95% confidence interval. Bolded = significant mean difference ($p<0.05$)

5.2 Figures: Aim 1 and Aim 2

Figure 1. CONSORT diagram

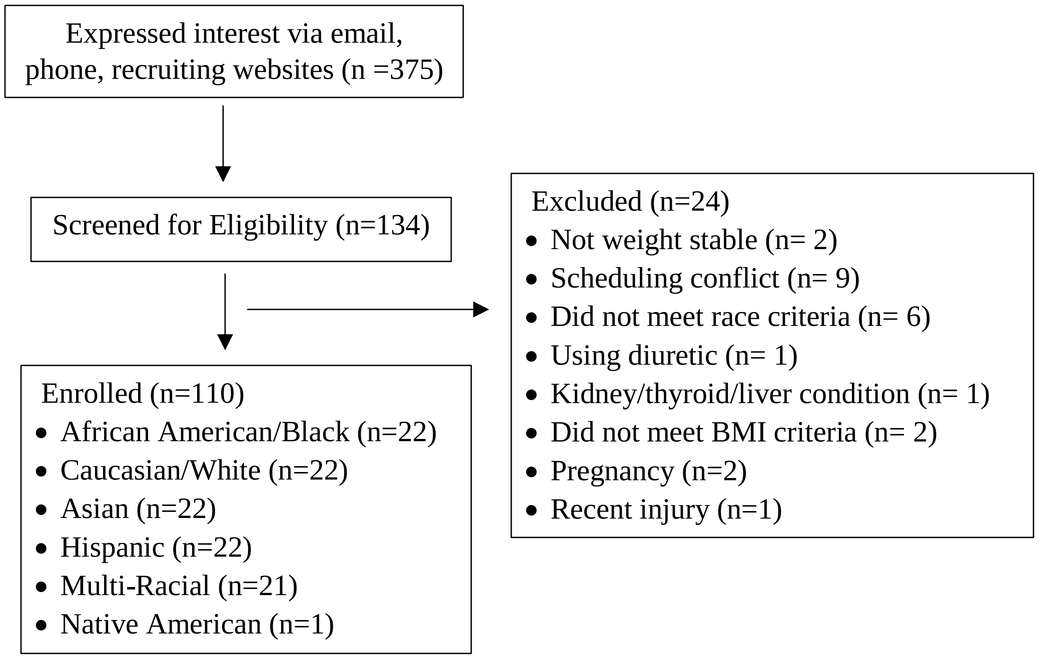
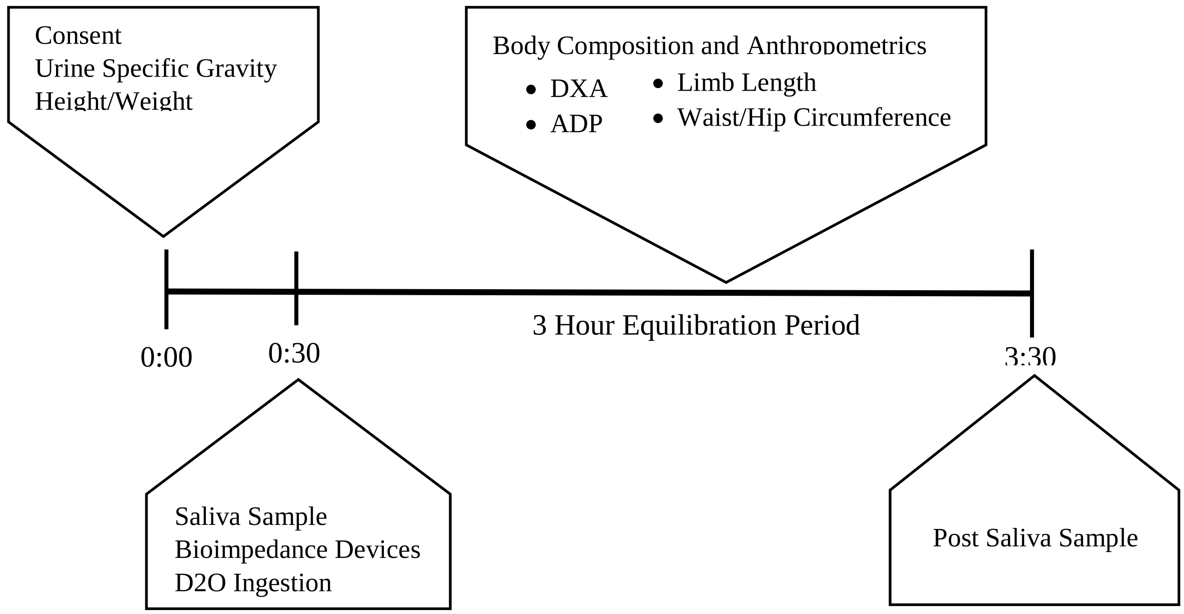


Figure 2 Aim 1 study design



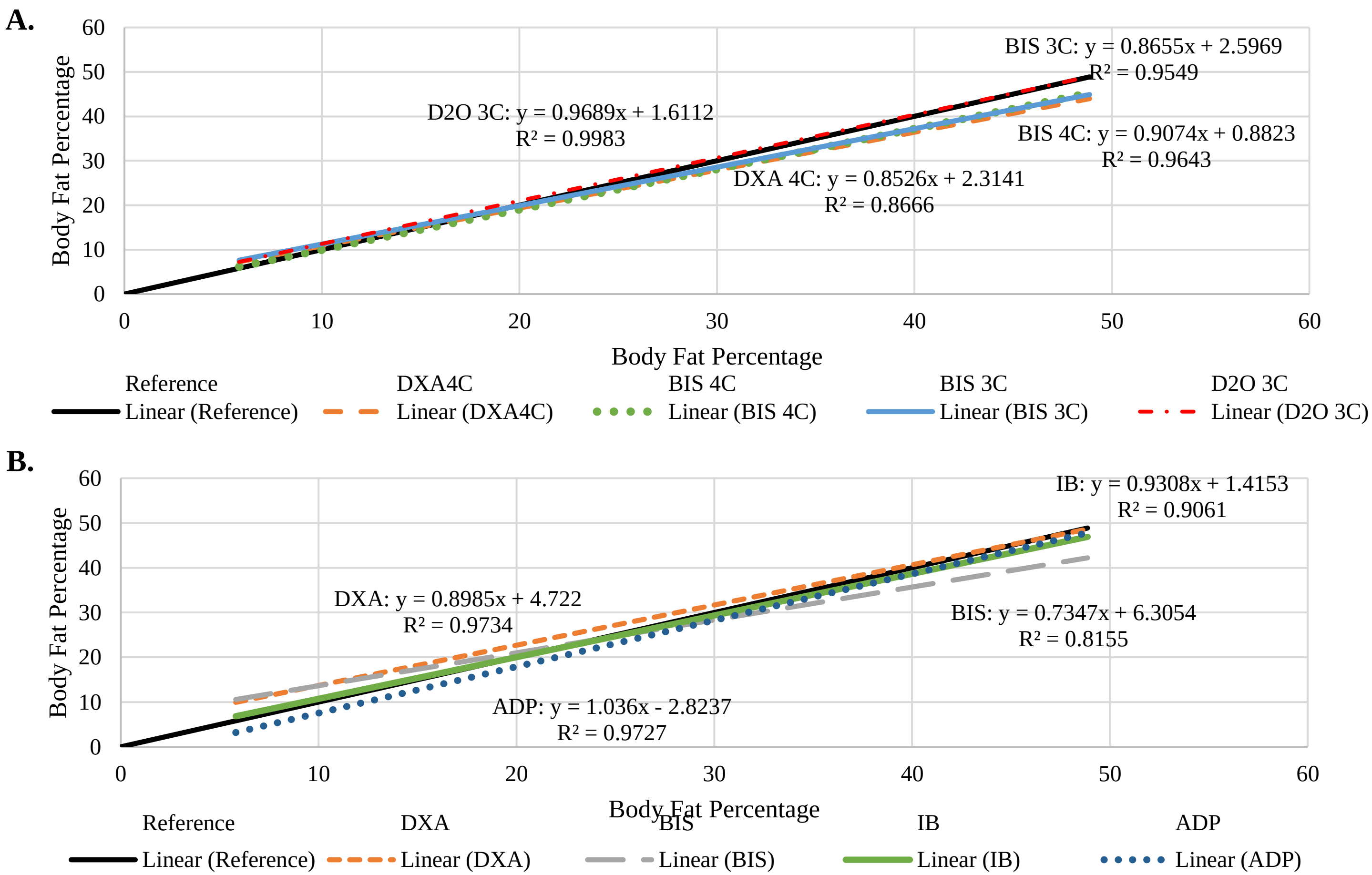


Figure 3. Simple regression analysis of total sample (n=110) for body composition models compared to reference 4C criterion. A) Multi-compartment model regressions (BIS 4C Slope: $p<0.001$, Intercept: $p=0.056$; BIS 3C Slope: $p<0.001$, Intercept: $p<0.001$; D_2O 3C Slope: $p<0.001$, Intercept: $p<0.001$; DXA-BV 4C Slope: $p<0.001$, Intercept: $p=0.008$). B) Regression analysis for total sample (n=110) for single device models (DXA Slope: $p<0.001$, Intercept: $p<0.001$; ADP Slope: $p<0.001$, Intercept: $p<0.001$; BIS Slope: $p<0.001$, Intercept: $p<0.001$; IB Slope $p<0.001$, Intercept: $p=0.074$). Significance indicates significantly different slope from 1 and intercept from 0.

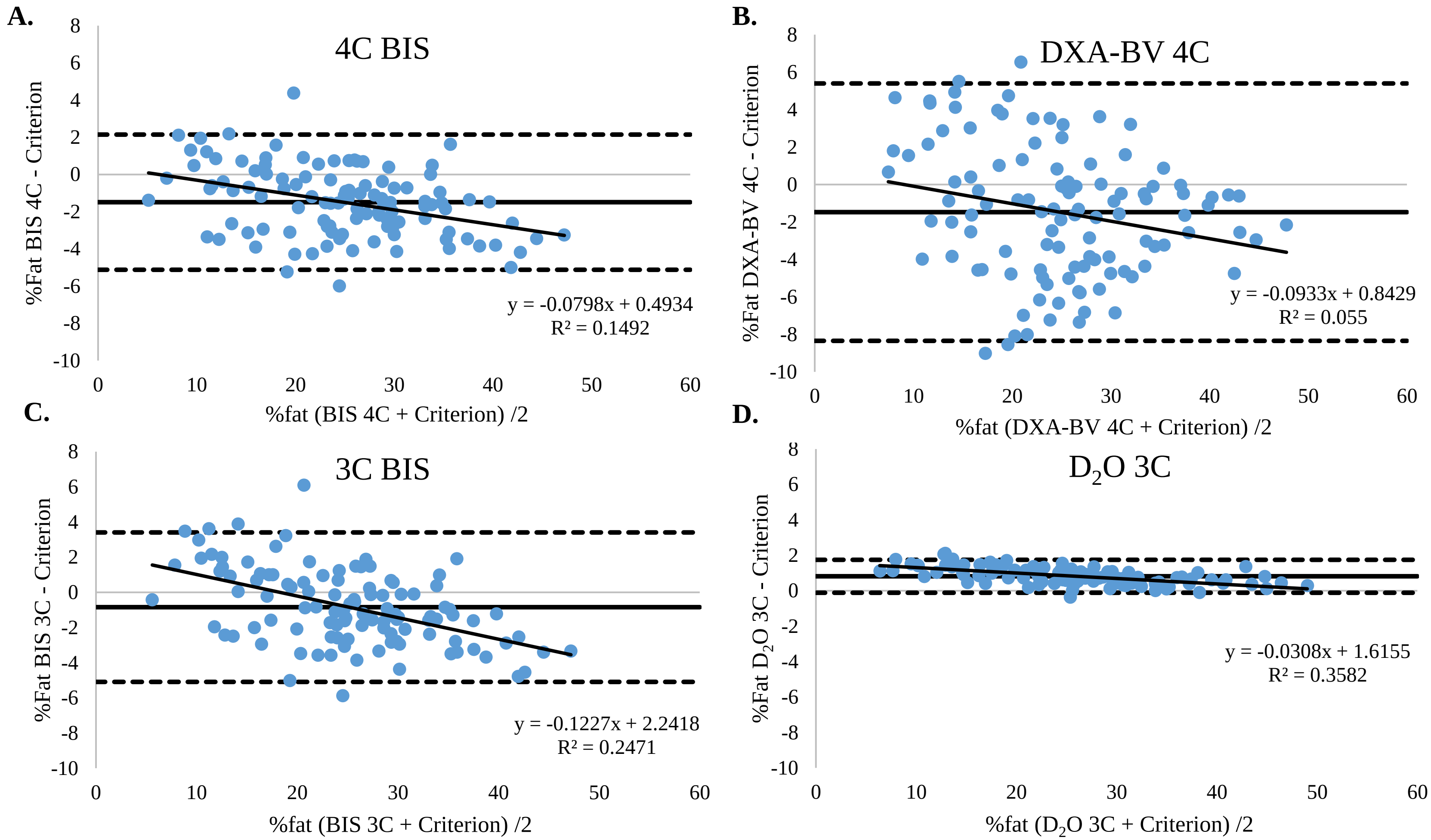


Figure 4. Bland Altman plot analyses and regression for multi-compartment models body fat percentage estimates. A) Bioelectrical impedance spectroscopy 4C model (95% Limits of Agreement [LOA] = -5.1 – 2.4 %; Mean difference [MD] = -1.5 %; Regression equation: $p<0.001$); B) Dual energy X-ray absorptiometry derived body volume 4C model (LOA = -8.3 – 5.4 %; MD = -1.5 %; Regression equation: $p=0.014$); C) Bioelectrical impedance spectroscopy 3C model (LOA = -5.1 – 3.4 %; MD: -0.8 %; Regression equation: $p<0.001$); D) Deuterium dilution 3C model (LOA = -0.1 – 1.7 %; MD = 0.8 %; Regression equation: $p<0.001$).

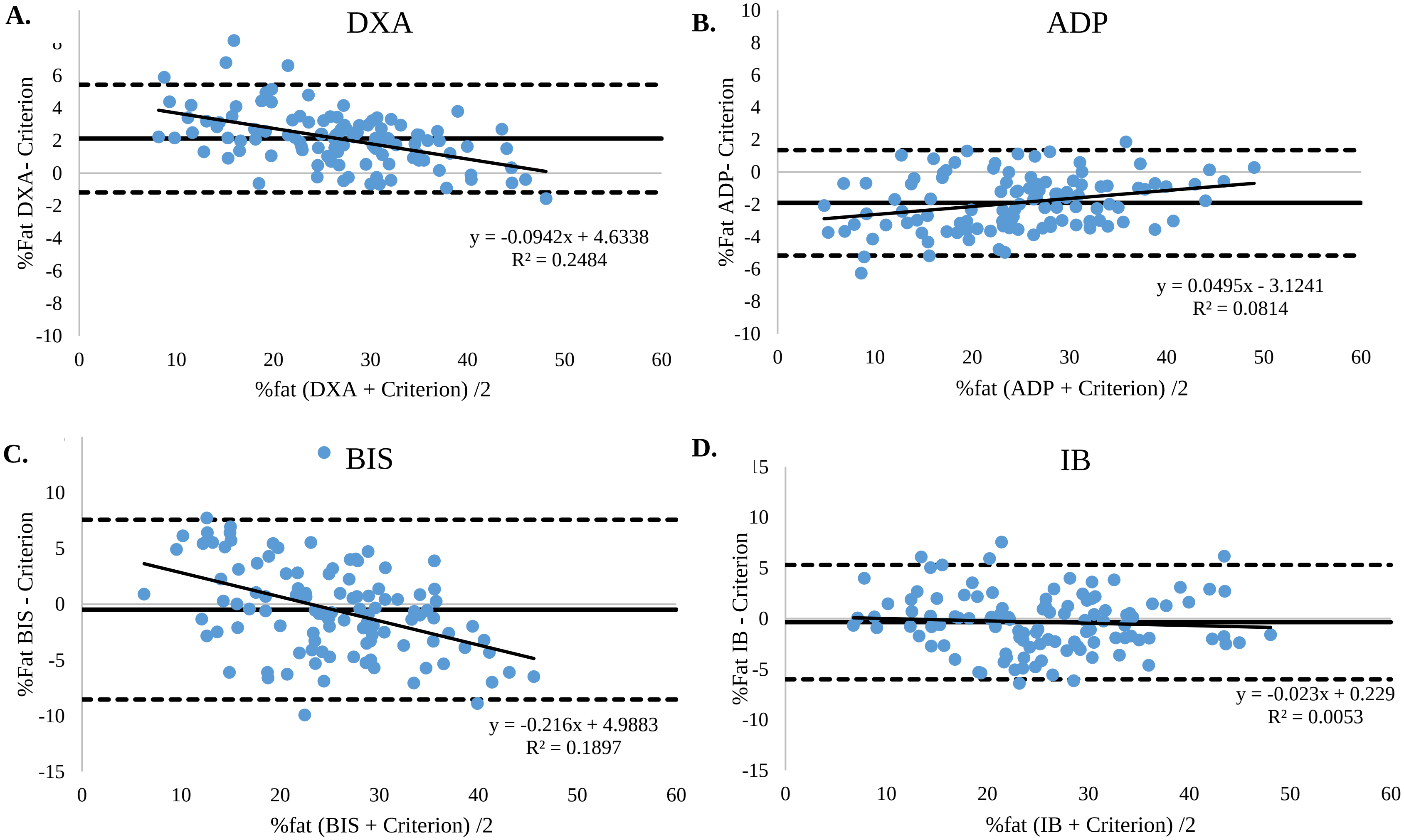


Figure 5. Bland Altman plot analyses and regression for single device body fat percentage estimates. A) Dual energy X-ray absorptiometry (95% Limits of Agreement [LOA] = -1.2 – 5.4 %; Mean difference [MD] = 2.1 %; Regression equation: p<0.001); B) Air displacement plethysmography (LOA = -5.1 – 1.4 %; MD = -1.9 %; Regression equation: p=0.003); C) Bioelectrical impedance spectroscopy (LOA = -8.5 – 7.6 %; MD = -0.5 %; Regression equation: p<0.001); D) Inbody (LOA = -6.0 – 5.3 %; MD = -0.4 %; Regression equation: p=0.449).

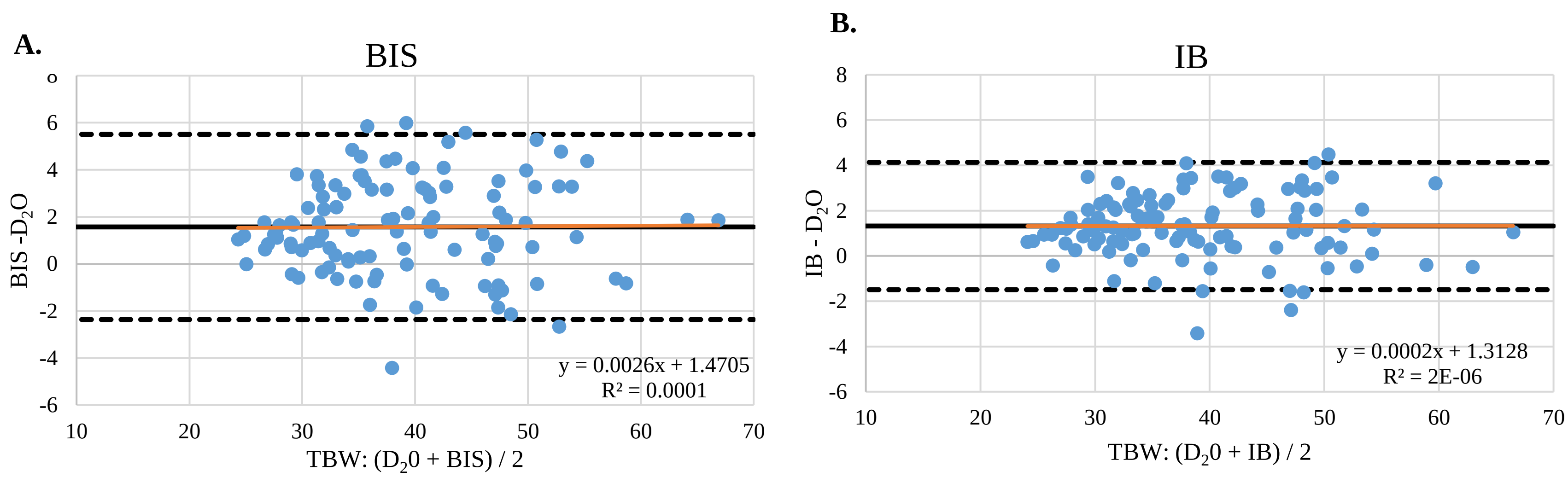


Figure 6. Bland-Altman analyses for Impedimed total body water measures (95% Limits of Agreement [LOA] = -2.36 – 5.51 L; Constant error [CE] = 1.57 L; Regression equation (orange): p=0.991); B) Bland-Altman analyses for Inbody total body water measures (LOA = -1.49 – 4.13 L; CE = 1.32 L; Regression equation (orange): p=<0.999)

CHAPTER VI: AIM 3 AND AIM 4 SUMMARY OF RESULTS

6.1 Aim 3 Results

For arm and leg FFM, the IB and BIS estimates were significantly different from the DXA measures for all races ($p<0.05$), except for IB leg FFM measure in multi-racial individuals (IB: 8.6 ± 1.9; DXA: 8.4 ± 2.2; $p=0.123$). For the thigh, BIS values were significantly different from DXA for the total sample and Hispanic individuals ($p<0.05$), but not significantly different for Asian ($p=0.060$), African American/Black ($p=0.209$), Caucasian/White ($p=0.304$) and Multi-Racial ($p=0.057$) samples. Mean (SD), mean difference (MD) and 95% CI data presented in Table 11. Bland-Altman plot and regression analyses (Figures 8-10) demonstrate individual variability for the total sample. Proportional bias existed for all measures ($p<0.01$). For IB and BIS, arm and leg estimates were underestimated for individuals with greater FFM; thigh BIS measures were overestimated for individuals with greater FFM. Limits of agreement were narrower for the IB compared to BIS for both arm and leg measures (Figure 8-9).

Arm

For the total sample and in each individual race/ethnicity, BIS demonstrated higher TE (0.46-0.67 kg) compared to IB (0.26-0.40 kg). For the total sample, Asian, African American/Black, SEE and R^2 was similar between devices (Table 10). For Hispanic and Multi-Racial individuals, IB demonstrated higher SEE (0.20 – 0.26 kg) and lower R^2 (0.93-0.95) than BIS (SEE=0.11 – 0.14 kg; R^2=0.98). For Caucasian/White individuals, IB demonstrated lower SEE (0.22 kg) and higher R^2 (0.94) compared to BIS (SEE=0.32 kg; R^2=0.87).

Using Caucasian/White as the comparator, for IB, TE was lower for all other racial groups (Table 10). SEE was higher for African American/Black and Multi-racial individuals, similar to Hispanic participants and lower for Asian participants. For BIS, TE was lower for Asian and Hispanic samples, similar for Multi-Racial individuals and higher for African American/Black. SEE was lower for all other races.

Results demonstrated arm FFM accounts for approximately 5% of total body FFM, therefore SEE/TE subjective rating values (According to Heyward and Wagner [49]) were adjusted to match 5% of TE/SEE expected from the total body. For excellent to fair agreement, SEE/TE values should be between 0.09-0.23 kg. TE values were not acceptable for IB or BIS for any race/ethnicity. According to SEE, IB arm FFM measures compared to DXA may be good for Asian individuals, fair for Hispanic and Caucasian, but poor for all other races/ethnicities. For BIS, SEE values were excellent for Hispanic, Multi-Racial and Asian participants, fair for African American/Black and poor for Caucasian.

Leg

For the total sample, and in each individual race/ethnicity BIS demonstrated significantly higher TE (1.5 - 2.6 kg) compared to IB (0.62 – 0.99 kg). For the total sample, Asian, Hispanic and Multi-Racial participants, SEE and R^2 was similar between devices (Table 10). For African American/Black individuals, IB demonstrated higher SEE (0.61 kg) and lower R^2 (0.93) than BIS (SEE=0.49 kg; R^2=0.95); for Caucasian/White individuals, IB demonstrated lower SEE (0.72 kg) and higher R^2 (0.86) compared to BIS (SEE=0.93, R^2=0.77).

With Caucasian/White as the comparator, for the BIS, TE was lower for Asian and Hispanic, similar as Multi-racial and higher for African American/Black individuals; SEE was lower for all other races. For IB, TE and SEE were lower for all races.

Results demonstrated leg FFM accounts for approximately 14.7% of total body FFM; the same procedure to adjust SEE/TE for arm were completed for the leg. For excellent to fair agreement, SEE/TE values should be between 0.26-0.66 kg. Therefore, IB and BIS, leg FFM TE values were poor for all races, with the exception of IB leg estimates for Hispanic individuals which were fair. According to SEE values, Asian and Hispanic participants demonstrated good to very good agreement for both IB and BIS, Caucasian/White individuals results were poor, and Multi-racial and African American/Black individuals were fairly good to fair for both IB and BIS.

Thigh

Using Caucasian/White as the comparator, for the BIS, TE and SEE were lower for Asian and Hispanic and higher for Black and Multi-racial individuals (Table 10).

Results demonstrated thigh FFM accounts for approximately 8.6% of total body FFM; the same procedure to adjust SEE/TE for arm were completed for the thigh. For excellent to fair agreement, SEE/TE values should be between 0.15-0.39 kg. Therefore, thigh BIS FFM validity was poor according to both SEE and TE for all races.

Table 10. Validity statistics comparing the segmental FFM values between DXA and the Inbody (IB) and Impedimed (BIS)

	Total		**Asian**		**Black**		**White**		**Hispanic**		**Multi-Racial**	
	IB	*BIS*	*IB*	*BIS*	*IB*	*BIS*	*IB*	*BIS*	*IB*	*BIS*	*IB*	*BIS*
Arm												
TE (kg)	0.33	0.56	0.26	0.46	0.31	0.67	0.40	0.60	0.31	0.47	0.33	0.60
SEE (kg)	0.23	0.22	0.16	0.15	0.26	0.22	0.22	0.32	0.20	0.11	0.26	0.14
R^2	0.95	0.95	0.96	0.97	0.95	0.96	0.94	0.87	0.95	0.98	0.93	0.98
Leg												
TE (kg)	0.80	2.00	0.83	1.52	0.82	2.56	0.99	2.10	0.62	1.62	0.67	2.09
SEE (kg)	0.70	0.70	0.45	0.46	0.61	0.49	0.72	0.93	0.39	0.43	0.61	0.63
R^2	0.89	0.89	0.91	0.91	0.93	0.95	0.86	0.77	0.96	0.96	0.92	0.91
Thigh												
TE (kg)	-	1.43	-	1.36	-	1.62	-	1.44	-	1.04	-	1.64
SEE (kg)	-	0.76	-	0.49	-	0.83	-	0.72	-	0.63	-	0.78
R^2	-	0.77	-	0.77	-	0.74	-	0.70	-	0.79	-	0.76

FFM = fat free mass; DXA = dual energy X-ray absorptiometry; IB = Inbody; BIS = Impedimed; TE = total error; SEE = standard error of the estimate

Table 11. Segmental fat-free mass values from DXA, Inbody and BIS. Presented as mean ± standard deviation, mean difference and 95% confidence intervals.

	Total	Asian	Black	White	Hispanic	Multi-Racial
Arm FFM (kg)						
DXA Mean (SD)	2.73 (0.98)	2.38 (0.86)	3.20 (1.12)*	2.78 (0.89)	2.46 (0.89)	2.90 (0.98)
IB Mean (SD)	2.96 (0.91)	2.58 (0.76)	3.36 (1.01)	3.12 (0.85)	2.70 (0.86)	3.11 (0.91)
BIS Mean (SD)	2.22 (0.82)	1.98 (0.67)	2.59 (0.91)	2.28 (0.87)	2.02 (0.73)	2.29 (0.80)
MD_1 [CI]	**0.23** [0.19, 0.27]	**0.19** [0.11, 0.27]	**0.16** [0.04, 0.28]	**0.34** [0.24, 0.43]	**0.24** [0.15, 0.33]	**0.21** [0.09, 0.33]
MD_2 [CI]	**-0.50** [-0.55, -0.45]	**-0.40** [-0.50, -0.30]	**-0.61** [-0.74, -0.48]	**-0.50** [-0.65, -0.36]	**-0.43** [-0.51, -0.35]	**-0.56** [-0.66, -0.45]
Leg FFM (kg)						
DXA Mean (SD)	7.81 (2.12)	6.93 (1.49)	8.89 (2.29) #	7.97 (1.94)	6.98 (2.05)	8.39 (2.19)
IB Mean (SD)	8.16 (1.82)	7.64 (1.47)	8.50 (1.80)	8.67 (1.79)	7.46 (1.94)	8.62 (1.85)
BIS Mean (SD)	5.96 (1.56)	5.48 (1.31)	6.45 (1.62)	6.08 (1.65)	5.49 (1.51)	6.42 (1.50)
MD_1 [CI]	**0.35** [0.22, 0.49]	**0.70** [0.50, 0.90]	**-0.39** [-0.72, -0.06]	**0.70** [0.39, 1.02]	**0.48** [0.30, 0.66]	0.23 [-0.07, 0.52]
MD_2 [CI]	**-1.83** [-1.99, -1.67]	**-1.45** [-1.66, -1.24]	**-2.44** [-2.79, -2.09]	**-1.89** [-2.30, -1.48]	**-1.49** [-1.78, -1.20]	**-1.91** [-2.32, -1.50]
Thigh FFM (kg)						
DXA Mean (SD)	4.62 (1.49)	3.93 (1.02)	5.50 (1.62) #	4.72 (1.32)	4.07 (1.38)	4.99 (1.58)
BIS Mean (SD)	5.12 (2.39)	4.47 (2.07)	5.94 (2.76)	5.04 (2.34)	4.61 (1.89)	5.68 (2.68)
MD [CI]	**0.49** [0.24, 0.75]	0.54 [-0.02, 1.11]	0.44 [-0.27, 1.15]	0.32 [-0.31, 0.96]	**0.54** [0.13, 0.94]	0.69 [-0.02, 1.40]

FFM = fat free mass; DXA = dual energy X-ray absorptiometry; IB = Inbody; BIS = Impedimed; SD = standard deviation; MD_1 = mean difference between DXA and IB; MD_2 = mean difference between DXA and BIS; CI = 95% Confidence interval of the difference. Bolded = significant mean difference ($p<0.05$). *Significantly different than Asian; #Significantly different than Asian and Hispanic

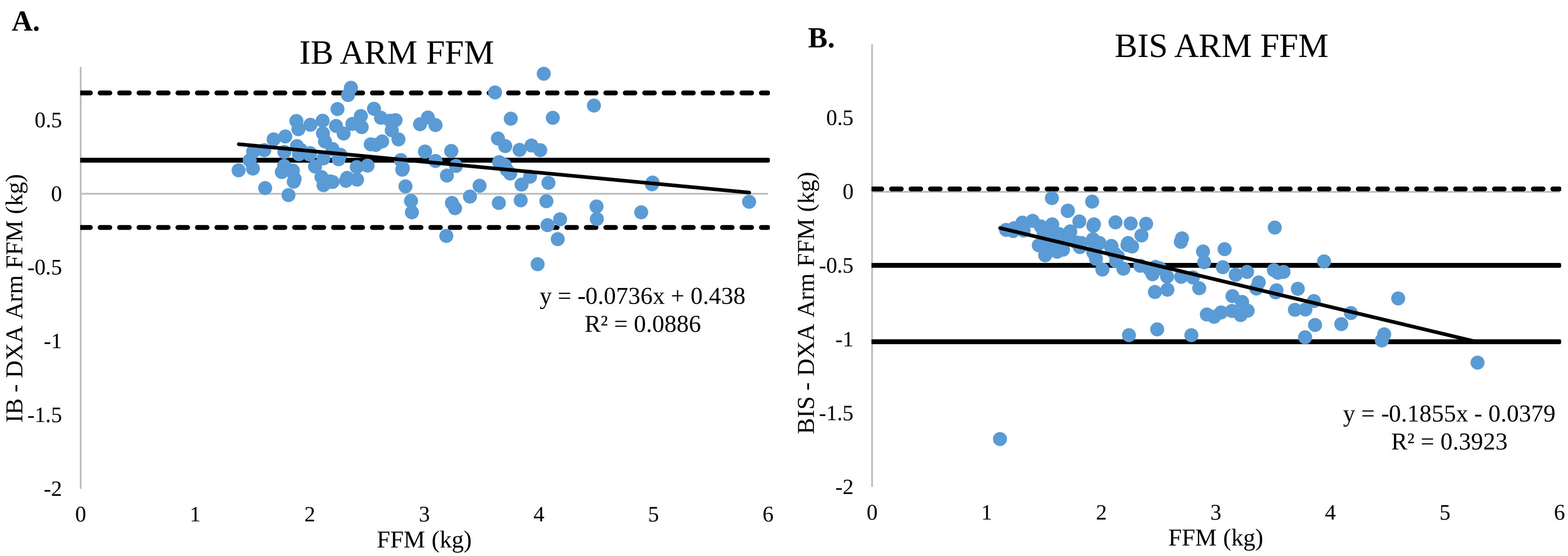


Figure 7. Bland-Altman analyses for A) arm Inbody measures (95% Limits of Agreement [LOA] = -0.23 - 0.68 kg; Constant error [CE] = 0.23 kg; Regression equation: p=0.002; B) arm BIS (LOA = -1.02 - 0.02 kg; CE = -0.50 kg; Regression equation: p <0.001)

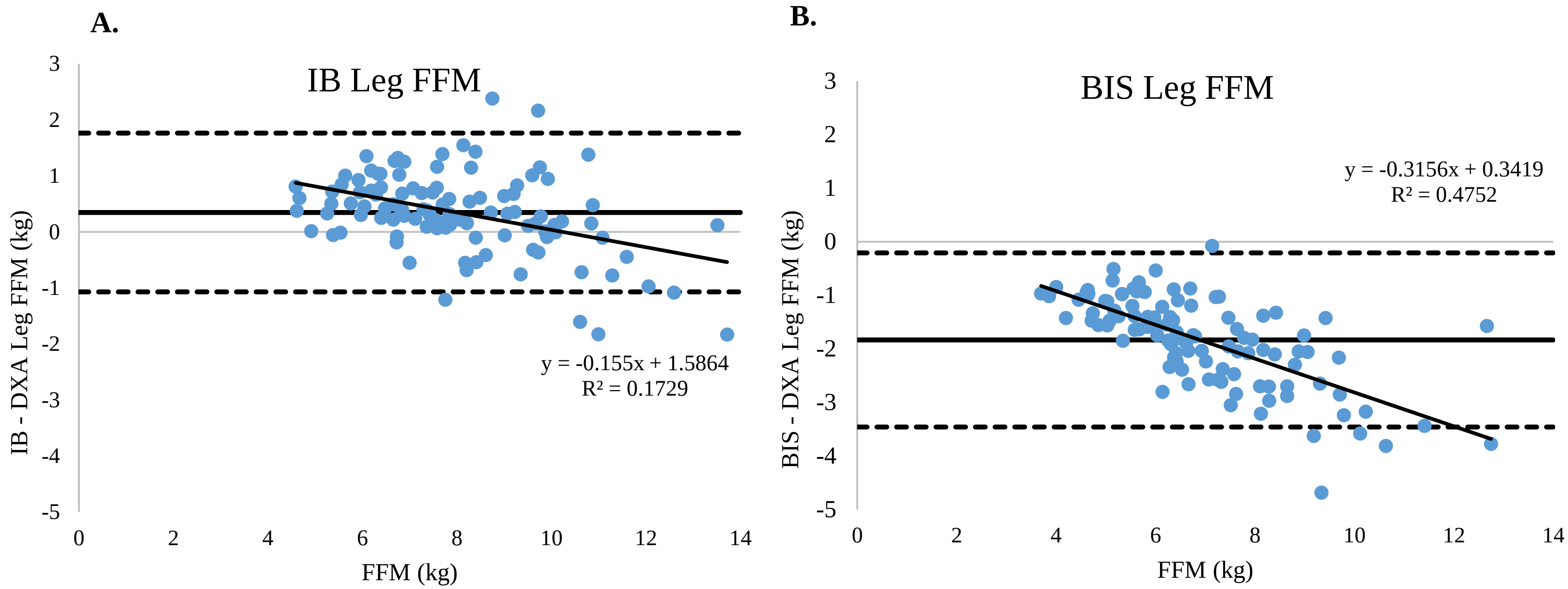


Figure 8. Bland Altman plot analyses for A) leg Inbody measures (95% Limits of Agreement [LOA] = -1.07 – 1.76 kg; Constant error [CE] = 0.35 kg; Regression equation: p=0.002; B) leg BIS (LOA = -3.46 - -0.21 kg; CE = -1.83 kg; Regression equation: p <0.001)

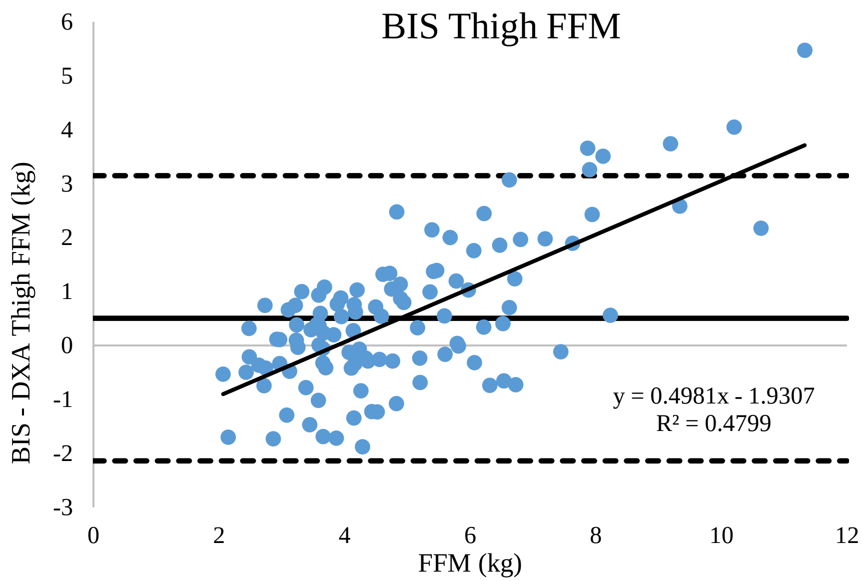


Figure 9. Bland-Altman analyses for thigh BIS measures (95% Limits of Agreement [LOA] = -2.14 – 3.15 kg; Constant error [CE] = 0.50 kg; Regression equation: $p<0.001$).

6.2 Aim 3 Discussion

For segmental measures of FFM, the validity of estimates from the Inbody 770 and Impedimed SFB7 compared to DXA varied by segment and race/ethnicity. Considering all validity statistics, our hypotheses that 1) BIS device measures would be valid and 2) IB measures would not be valid for African American/Black, Hispanic, Asian and Multi-racial participants were not supported. For arm and leg FFM, the IB and BIS estimates were significantly different from the DXA measures for all races ($p<0.05$), except for IB leg FFM measure in multi-racial individuals. Additionally, TE was not acceptable (fair to poor) for either device in a multi-ethnic sample for arm and leg measures. However, the IB produced lower TE compared to the BIS. Compared to the Caucasian/White sample, BIS estimates in Asian and Hispanic individuals demonstrated more valid results, Multi-Racial showed similar results and African American/Black estimates demonstrated greater TE. For IB, TE was higher in Caucasian/White individuals compared to all other races for arm and leg measures. According to SEE, both IB and BIS produced good to excellent agreement with DXA for arm and leg measures in Hispanic and Asian individuals. For thigh estimates, although significant mean differences between BIS and DXA were only observed for the total sample and Hispanic individuals, TE and SEE values demonstrated BIS is not a valid device for thigh measures compared to DXA for any race.

Results presented are similar to previous literature comparing multi-frequency bioimpedance devices (Inbody 720) measures to DXA [18,33,107]. Previous studies have primarily evaluated validity in collegiate athletes [33,107] and resistance-trained individuals [116]. In male and female athletes, validity of bioimpedance estimates of FFM in the arms (TE: 0.40 – 1.68 kg; SEE: 0.32 – 1.00 kg) and legs (TE: 1.38 – 5.84 kg; SEE: 1.13 – 3.17 kg) have been fair to poor [18,33,107]. Error was significantly greater for male athletes and individuals with greater FFM (i.e.

collegiate football athletes). Similarly, proportional bias analyses in the current study demonstrated that individuals with greater FFM were more significantly underestimated for arms and legs with both BIS and IB devices. In an older sample of Caucasian adults (61.2 - 63.5 yrs), one study reported small mean differences between right arm (0.2 kg) and right leg (0.6 kg) estimates from bioimpedance and DXA measures, potentially as a result of lower FFM [67].

Previous literature evaluating multi-frequency bioelectrical impedance spectroscopy devices (similar to Impedimed) for measures of segmental FFM have predominately been in elderly (65-85 yrs) [157,159] and clinical populations (i.e. hemodialysis) [59]. These studies have utilized regression analyses incorporating BIS estimates (i.e. intracellular water) and population characteristics such as age, weight and sex to evaluate thigh muscle cross sectional area [157] and limb muscle mass [59] compared to magnetic resonance imaging. Although, studies have reported good agreement (R^2=0.86-0.933), direct comparisons to our results are difficult due to population differences and the use of multiple regression, as opposed to estimating FFM directly from TBW measures, like the current study.

Based on results reported in our study and previous literature, IB and BIS estimates of segmental FFM may not be valid in a multi-ethnic sample. Hispanic and Asian individuals reported the lowest SEE values, however, TE was high for all races/ethnicities for arm, leg and thigh estimates. Therefore, if segmental FFM estimates are a key outcome in a diverse population, DXA may be recommended over bioelectrical impedance devices.

6.3 Aim 4 Results

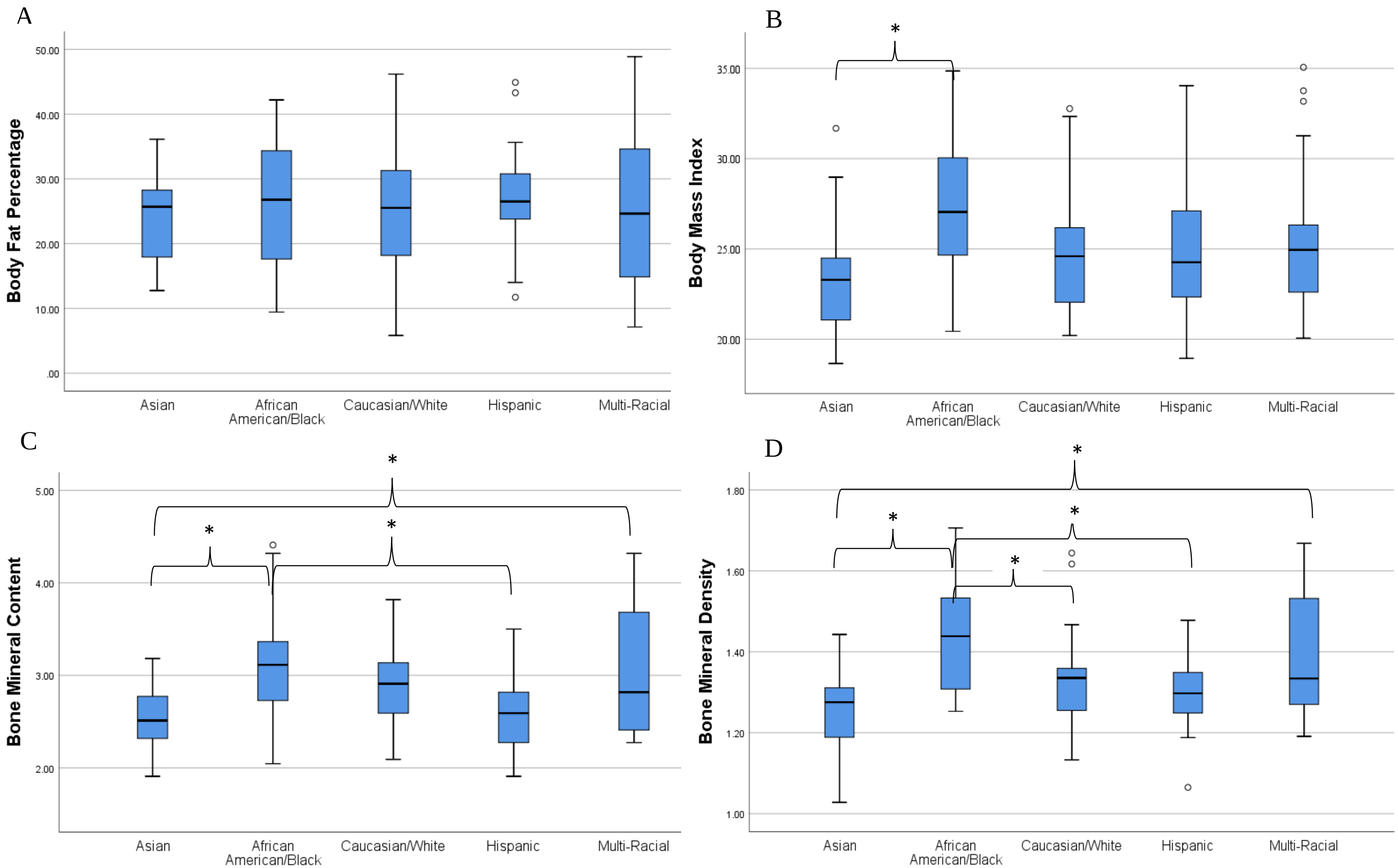
Results (Table 12, Figure 11) demonstrated that there were significant body composition differences between races/ethnicities for BMI ($p=0.004$), BMC ($p=0.001$), BMD ($p<0.001$), and FFM ($p=0.030$). Tukey HSD post-hoc analyses indicated there was a significant mean difference for BMI between African American/Black and Asian individuals (MD ± SE: 4.6 ± 1.1 kg/m^2; CI [1.37, 7.74], p=0.001); for BMC between African American/Black and Asian (MD ± SE: 0.59 ± 0.17 kg; CI [0.12, 1.05], p=0.006) and Hispanic (MD ± SE: 0.53 ± 0.17 kg; CI [0.06, 0.99], p=0.018) and Multi-Racial and Asian (MD ± SE: 0.53 ± 0.17 kg; CI [0.06, 1.00], p=0.020); for BMD between African American/Black and Asian (MD ± SE: 0.19 ± 0.04 g/cm^2; CI [0.08, 0.30], p<0.001), Hispanic (MD ± SE: 0.15 ± 0.04 g/cm^2; CI [0.04, 0.26], p=0.001) and White (MD ± SE: 0.12 ± 0.04 g/cm^2; CI [0.01, 0.23], p=0.018) and between Multi-Racial and Asian (MD ± SE: 0.14 ± 0.04 g/cm^2; CI [0.03, 0.25], p=0.005); for FFM, post-hoc analyses revealed no significant mean differences between groups (p=0.056-1.00). There were no mean differences for FM (p=0.232), %fat (p=0.813), VAT (p=0.847), Android:Gynoid ratio (p=0.574), or Waist:Hip ratio (p=0.778).

For muscle characteristics, there was a significant difference between races/ethnicities for MV (p=0.046); post-hoc analyses demonstrated a significant mean difference between African American/Black and Hispanic individuals (MD ± SE: 143.1 ± 50.7, CI [2.3, 283.8], p=0.044). No differences were observed for any other muscle characteristic variable (mCSA: p=0.173; EI: p=0.930; PA: p=0.567; FL: p=0.826; MT: p=0.828).

Table 12. Body composition and muscle characteristics stratified by race/ethnicity. Presented as unadjusted Mean (Standard deviation).

	Asian	**Black**	**White**	**Hispanic**	**Multi-Racial**
Body Composition					
A/G Ratio	0.97 (0.29)	0.97 (0.20)	0.88 (0.26)	1.00 (0.28)	0.93 (0.25)
BMC (kg)	2.5 (0.3) [#]	3.1 (0.6) [+]	2.9 (0.6)	2.6 (0.4)	3.1 (0.7)
BMD (g/cm^2)	1.26 (0.11)	1.45 (0.13) [1]	1.33 (0.13)	1.30 (0.09)	1.40 (0.16) [2]
BMI (kg/m^2)	23.1 (3.2)*	27.7 (4.4)	24.9 (3.4)	25.1 (3.7)	25.7 (4.3)
FFM (kg)	49.2 (10.1)	59.3 (13.7)	55.8 (12.2)	50.13 (12.4)	56.8 (12.8)
FM (kg)	15.5 (4.4)	21.5 (9.4)	18.1 (8.8)	18.7 (7.6)	19.5 (11.1)
%fat	24.2 (6.6)	26.4 (9.5)	24.5 (10.6)	27.2 (8.4)	25.1 (11.7)
VAT (kg)	0.28 (0.39)	0.36 (0.32)	0.37 (0.53)	0.39 (0.39)	0.42 (0.31)
Muscle Characteristics					
EI (au)	110.9 (37.0)	115.0 (38.8)	109.2 (42.9)	116.2 (36.7)	106.6 (41.8)
FL (cm)	6.3 (0.8)	6.6 (0.9)	6.4 (0.9)	6.3 (1.0)	6.4 (0.8)
mCSA (cm^2)	24.0 (8.1)	28.4 (8.1)	24.7 (6.1)	23.0 (7.4)	25.4 (7.3)
MT (cm)	2.6 (0.5)	2.6 (0.5)	2.6 (0.4)	2.5 (0.5)	2.6 (0.5)
MV (cm^3)	474.7 (148.5)	598.9 (207.0)	486.2 (125.5)	455.8 (166.8)*	527.2 (182.0)
PA (°)	18.9 (4.0)	20.2 (3.6)	20.2 (2.3)	20.4 (3.4)	20.4 (3.6)

A:G = Android:Gynoid; BMC = bone mineral content; BMD = bone mineral density; BMI = body mass index; FFM = fat free mass; FM = fat mass; %fat = body fat percentage; VAT = visceral adipose tissue; EI = echo intensity; FL = fascicle length; mCSA= muscle cross sectional area; MT = muscle thickness; MV = muscle volume; PA = pennation angle; *significantly different than Black (BMI, MV: $p<0.05$). [1]Significantly different than Asian, Hispanic, White (BMD: $p<0.05$); [2]Significantly different from Asian (BMD: $p<0.05$); [+] Significantly different from Asian and Hispanic (BMC: $p<0.05$); [#] significantly different from Multi-Racial (BMC: $p<0.05$)


A
Body Fat Percentage
50.00
40.00
30.00
20.00
10.00
.00
Asian
African American/Black
Caucasian/White
Hispanic
Multi-Racial
B
*
Body Mass Index
35.00
30.00
25.00
20.00
Asian
African American/Black
Caucasian/White
Hispanic
Multi-Racial
C
*
*
*
Bone Mineral Content
5.00
4.00
3.00
2.00
Asian
African American/Black
Caucasian/White
Hispanic
Multi-Racial
D
*
*
*
*
Bone Mineral Density
1.80
1.60
1.40
1.20
1.00
Asian
African American/Black
Caucasian/White
Hispanic
Multi-Racial

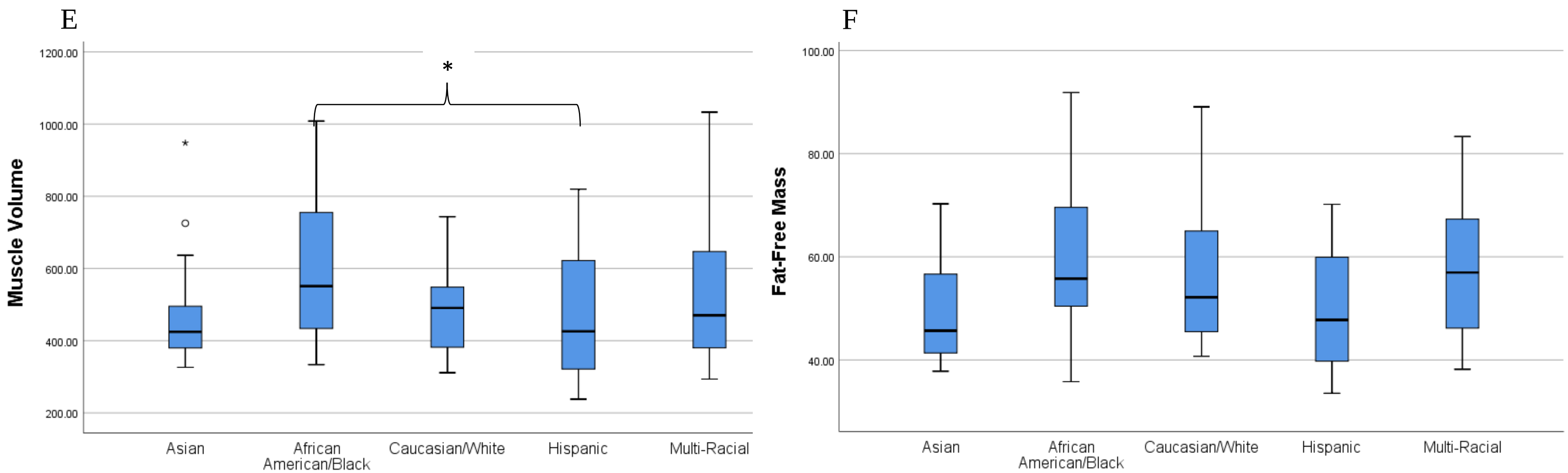


Figure 10. Box Plot Analysis for A) body fat percentage (%), B) body mass index (kg/m^2), C) bone mineral content (kg), D) bone mineral density (g/cm^2), E) muscle volume (cm^3), F) fat-free mass (kg). *significant mean difference ($p<0.05$) .

6.4 Aim 4 Discussion

The present study observed differences between races/ethnicities in bone estimates and muscle volume. Specifically, BMC was greater in African American/Black participants (3.1 ± 0.6 kg) compared to Asian (2.5 ± 0.3 kg) and Hispanic (2.6 ± 0.4 kg) individuals and Multi-racial (3.1 ± 0.7 kg) participants had greater BMC compared to Asians. For BMD, African American/Black participants (1.45 ± 0.13 g/cm^2) had greater values compared to all races (1.26 – 1.33 g/cm^2), except Multi-racial (1.40 ± 0.16 g/cm^2) which had significantly greater BMD than Asian. Muscle volume was greater in African American/Black individuals (598 ± 207 cm^3) compared to Hispanic participants (455.8 ± 166.8 cm^3). Although not significant, FFM differences between African American/Black participants (59.3 ± 13.7 kg) and Asian individuals (49.2 ± 10.1 kg) trended toward significance (p=0.056). There were no significant differences between races/ethnicities for total body %fat or FM, and no differences in fat distribution variables (VAT, A:G ratio, W:H ratio), even though BMI was found to be greater in African American/Black (27.7 ± 4.4 kg/m^2) compared to Asian (23.0 ± 3.2 kg/m^2) participants. Additionally, no differences between races/ethnicities were observed for any muscle architecture (FL, PA), quality (EI) or size (mCSA, MT) variables, with the exception of MV.

Previous research comparing African American/Black (AA) and Caucasian/White (W) males and females have demonstrated a similar relationship for BMC (AA: 3.02-3.11 kg; W: 2.71-2.72 kg) [139] and BMD (AA: 1.25-1.35 g/cm^2; W: 1.16 – 1.25 g/cm^2) [82,139] as the current study. A study evaluating racial differences reported significantly lower BMC in Chinese males and females (2.34 – 3.04 kg) compared to Caucasians (2.82 – 3.46 kg), but no significant difference in BMD (Chinese: 1.04 – 1.06 g/cm^2; Caucasian: 1.03 – 1.06 g/cm^2)[152]. Although the present study did not observe a difference between Asian and Caucasian individuals for bone estimates, Asian participants reported the lowest BMC and BMD values compared to other racial/ethnic groups.

As BMC is a component of FFM, greater BMC may have contributed to the greater FFM estimates observed in African American/Black participants. The mean difference of 10.1 kg compared to Asian individuals suggests the greater FFM is also related to larger amounts of lean soft tissue for African American/Black participants. A previous study utilizing National Health and Nutrition Examination Survey (NHANES) DXA data reported African American/Black males had a greater lean mass index, specifically in the legs, compared to Hispanic and Caucasian/White males [53]. Our results are similar to a previous study evaluating FFM index (FFMI) across age and race in adults, which observed African American/Black individuals had the greatest FFMI and Asians had the lowest, regardless of age and sex [76].

Racial differences in total FM, %fat and fat distribution were not significant in the present study. Differences between races/ethnicities are inconsistent in previous studies with some reporting no racial differences in %fat [56,104], VAT [110,134] and W:H ratio [83,135], and other studies observing greater total and regional FM (i.e. leg, trunk, arm in African American/Black and Hispanic individuals compared to Caucasian/White [53,78] and less VAT in African American/Black participants compared to Caucasian/White [52]. Asian individuals have more consistently been reported to have greater VAT [83,120] compared to other races, which was not observed in the current study. A previous study that differentiated between Chinese Americans and South Asian individuals saw South Asians had significant greater VAT [120]; combining all Asians in the present study (n=6 Southeast Asian, n=11 East Asian, n=5 South Asian) may have decreased our ability to observe differences in VAT.

For muscle characteristics, results from the present study are similar to previous investigations which have observed greater MV in African America/Black individuals [31,42]. A study utilizing MRI reported African Americans had the largest muscle size and Asians the smallest [42]. Although not significant in the present study, a previous study utilizing CT reported African Americans had greater MV compared to Caucasians in older adults [31]. Research evaluating racial/ethnic differences in muscle architecture is limited, however, similar to our results, a study evaluating FL and PA did not observe differences between African American and Caucasian individuals [1]. Evaluations of muscle quality and intramuscular fat have observed inconsistent results when utilizing ultrasound EI [78,79]; populations varied significantly between studies (overweight/sedentary vs. collegiate athletes) which may have contributed to the difference in results. Utilizing CT/MRI, fat infiltration differences between races have more consistently observed African Americans [80,110] and Asian [120] individuals have greater fat infiltration and lower muscle quality, particularly in older populations. Echo intensity measures via ultrasound may not be sensitive enough to detect racial differences in a broad sample (i.e. age: 18-45 yrs, %fat: 6-48%) like in the present study.

The relationship of fat distribution, FFM and muscle size, cardiometabolic health [121,164] and functional (i.e. strength) health [19,39] suggests these variables should be evaluated more thoroughly by race/ethnicity, age, sex and training status in a larger sample. Subsequent analyses should observe which variables are most related to cardiometabolic disease risk within each race/ethnicity as the relationship may vary across races/ethnicities.

CHAPTER VII: CONCLUSIONS

Based on the current study results, multi-compartment models, DXA, ADP and IB can be utilized in multi-ethnic samples and in each individual race/ethnicity to obtain highly valid results for both %fat and FFM. The most accurate estimates for all races/ethnicities were obtained from D2O 3C, BIS 4C, BIS 3C (TE = 0.9 – 2.4 %), followed by DXA, ADP, IB (TE= 2.5 – 2.9%), and then DXA-BV 4C and BIS (TE = 3.79 – 4.12%). Investigators should use caution when interpreting results from the DXA-BV 4C model and BIS. BIS estimates were not valid in African American/Black, Caucasian/White and Multi-racial samples. The sources of error (i.e. the BIS TBW estimate or the BV prediction) for the DXA-BV 4C model should be evaluated further. Mean estimates from 4C BIS, 3C BIS and ADP may underestimate %fat (1-2%) and overestimate FFM (~1 kg), respectively; while DXA may overestimate %fat (~2%).

The two multi-frequency bioelectrical impedance devices in the present study can produce valid TBW estimates compared to the D_2O criterion in a multi-ethnic sample and within each race/ethnicity. TBW estimates from the IB (TE = 1.6 – 2.4 L) may be more accurate compared to the BIS (2.3 – 3.0 L). Although both device estimates were considered valid, these devices may overestimate TBW by 1-2 L.

For segmental measures of FFM, TE was not acceptable (fair to poor) for either device in the multi-ethnic sample or within each individual race/ethnicity for arm, leg and thigh measures. Therefore, if segmental FFM estimates are a key outcome in a diverse population, DXA may be recommended over bioelectrical impedance devices.

The present study observed differences between races/ethnicities in bone estimates and muscle volume measured via ultrasound. BMC, BMD and MV were greatest in African American/Black participants (3.1 ± 0.6 kg, 1.45 ± 0.13 g/cm^2, 598 ± 207 cm^3, respectively) compared to Asian and Hispanic participants (BMC: 2.5 -2.6 kg; BMD: 1.26 – 1.30 g/cm^2; MV: 455.8 – 474.7 cm^3) No significant differences between races/ethnicities were observed for other body composition variables (%fat, FM, FFM, VAT) or muscle characteristic variables (FL, PA, EI, mCSA, MT). However, the relationship of fat distribution, FFM and muscle size to cardiometabolic health [121,164] and functional (i.e. strength) health [19,39], suggests these variables should be evaluated more thoroughly by race/ethnicity, age, sex and training status in a larger sample.

Overall, validity of the body composition models evaluated did not vary significantly between race/ethnicity; future studies should investigate the influence of sex, age and training status on the validity of body composition methods.

APPENDIX 1[1]

Validity of body composition methods across racial and ethnic populations

Introduction

The high rates of obesity, cardiovascular, and metabolic disease in minority populations [63,95,133] requires a reevaluation of our ability to assess and manage body composition effectively. According to the U.S. Census Bureau, within the next 40 years, over 50% of the U.S. population will consist of individuals who identify as a racial/ethnic minority. However, many previous body composition validation studies have not included minority populations or have failed to adequately report race/ethnicity of study participants. These omissions are important to consider as compartments of the body may vary depending on race and ethnicity [139], potentially leading to inaccurate assessments.

Body composition assessments were established to accurately estimate the various components of body mass such as fat tissue, lean soft tissue, bone mineral content, and total body water. Multi-compartment models are currently considered the gold standard for molecular-level body composition estimation and have the ability to account for multiple constituents, yielding more accurate estimates than simpler methods [50,148]. However, multi-compartment models require a minimum of two devices to measure additional compartments of the body and may not be the most feasible or practical technique. Therefore, single-device two-compartment (2C) models (i.e. air displacement plethysmography [ADP], bioelectrical impedance analysis [BIA], bioelectrical impedance spectroscopy [BIS], and hydrostatic weighing [HW]) and three-compartment (3C)

[1] This Appendix has been submitted for publication in Advances in Nutrition. The original citation is as follows: Blue MNM, Tinsley GM, Ryan ED, Smith-Ryan AE. Validity of body composition methods across racial and ethnic populations. Submitted to *Advances in Nutrition*. March 2020.

models (i.e. dual-energy X-ray absorptiometry [DXA]) are more commonly utilized to estimate fat mass (FM) and fat-free mass (FFM). Due to the measurement of fewer body compartments, several assumptions must be met for accurate estimates of body composition by these methods. Depending on the device, the validity of measures may be influenced by hydration, fat distribution, body proportions, and fat-free body density [36,50]. Investigations evaluating body composition in racial and ethnic minorities have observed differences in fat distribution (i.e. visceral vs. subcutaneous, intramuscular fat, trunk vs. limbs) [52,53,120], fat-free body density [139], and body proportions [139] between cohorts. Consequently, for racial/ethnic minority populations, estimates of body composition, especially by 2C models may not be valid. The purpose of this review was to comprehensively examine the validity of common body composition devices in multi-ethnic samples (i.e. samples including more than one race/ethnicity), African American/Black, Hispanic, Asian, and Native American populations. Results of this review may improve our ability to select the appropriate method (Appendix B) to accurately assess body composition in each racial/ethnic population potentially leading to better classification of obesity-related disease risk.

Assessment of Validity

Validity is often evaluated by a combination of statistical analyses with the primary aim of evaluating the difference and relationship between two measurements, with one typically serving as a criterion method. When evaluating group means, common statistical outcomes include mean difference (MD) ± standard deviation (SD), correlation coefficients (the relationship between the two scores; e.g., Pearson's r or the concordance correlation coefficient), the coefficient of determination (r^2; the amount of variance shared by the two outcomes), total error (TE; the average deviation of individual scores from the line of identity; also known as root mean square error and pure error), and standard error of the estimate (SEE; the degree of deviation of the individual data points around the line of best fit). Evaluation of whether the intercept and slope of the line of best fit significantly deviate from the line of identity (Intercept=0, Slope=1) is also common. For individual level differences, Bland-Altman analysis with the calculation of the 95% limits of agreement (LOA; representing the 95% likely reference range for the difference between method estimations) is often conducted. Simple linear regression analysis often accompanies the Bland-Altman analysis to determine if the level of agreement between methods varies based on the quantity of the variable being assessed (i.e., proportional bias). Therefore, for assessment of validity, this review will include all reported statistical procedures. For interpretation, we will follow the subjective rating outlined by Heyward and Wagner [49] (Table 1). Additionally, due to the limited data presenting validity of ADP and DXA in multi-ethnic samples, studies with small sample sizes (n=2-8) of minority individuals were included.

Validity of Dual Energy X-ray Absorptiometry

Dual energy X-ray absorptiometry is widely considered as a valid method for measuring body composition. Although few recent studies have explored the validity of DXA within racial and ethnic minority populations, DXA devices are commonly used in large-scale field-based studies (i.e. National Health and Nutrition Examination Survey) as well as in clinical and laboratory settings. DXA devices measure FM, lean soft tissue, and bone mineral based on the attenuation of a dual-photon energy low-dose X-ray beam. For valid measures, the DXA assumes constant soft tissue hydration, proper patient positioning and accurate proprietary algorithms estimating soft tissue in body compartments containing bone [36].

Validity within Multi-Ethnic Samples

In a multi-ethnic sample of 23 individuals (12 white, 3 black and 8 Puerto Rican), the DXA (Lunar DPX model) measures of FM demonstrated a MD of 1.51 ± 1.1 kg, technical error of 1.31

kg, SEE of 1.73 kg, and r^2 of 0.972 [148]. A follow up investigation in 14 Caucasian, 5 African American and 8 Puerto Rican participants found no significant difference in %fat estimates (MD: 0.54 ± 2.4 %, r=0.983) between DXA and the 5C model [146]. A study evaluating a fan bean DXA (Hologic, QDR 4500A) compared to a four compartment (4C) model included 6 African Americans in a sample of 58 participants ages 70-79 yrs and reported a SEE of 1.6 kg for measures of FFM, and a strong correlation (r=0.99) [136]. In a smaller sample, 13 participants, including 2 African Americans, reported a significant difference between the 4C body composition estimate of FFM (51.1 ± 12.4 kg) compared to fan beam (53.7 ± 12.9 kg) and pencil beam (48.4 ± 12.1 kg) DXA estimates [136]. In a college-age sample of 62 black and 110 white males and females there were no significant differences between DXA %fat and the 4C model (MD: 0.4 ± 2.9%, r=0.94, SEE=2.8%)[100]. In the multi-ethnic samples presented, DXA estimates demonstrated very good to excellent validity compared to multi-compartment models [50]. However, the representation of racial/ethnic minorities primarily only included African Americans and a small number of Puerto Ricans which accounted for between 10 – 47% of the sample.

Validity by Race/Ethnicity

An investigation evaluating Native American females ages 18-60 yrs, reported that DXA measures of %fat demonstrated good to very good validity compared to a 3C density model (r^2=0.785, SEE=3.28%, TE=3.27%) [51]. Conversely, in a study of 30 black males (19-45 yrs, BMI=18.9-40.5 kg/m^2), DXA and 4C %fat were not significantly different (MD=-0.28%), with SEE=2.26% and r=0.95 [140]. Additionally, DXA %fat was not significantly different from a 4C estimate (MD=0.2%) in 39 black males [22]. In a sample of 291 Asian (Chinese n=108, Malays n=76, Indian n=107) males and females ages 18-75 yrs and BMI between 16-40 kg/m^2, DXA estimates of %fat were underestimated compared to the 4C. Mean differences ranged between 2.1 - 2.5 %

for females and between 3.2 - 4.2 % for males within the three ethnic cohorts, with a moderate correlation between methods (F: r=0.62; M: r=0.56) [25]. To our knowledge, studies investigating the validity of DXA in Hispanic populations [105] and in larger multi-ethnic samples [155] have not been conducted in adults, with available data only in children ages 9-17 yrs. Future investigations should evaluate the validity of DXA, particularly in adult Hispanic/Latinx populations, as well as in cohorts including both sexes for black and Native American populations. The aforementioned studies investigating racial/ethnic minorities did not report any individuals identifying as two or more races; future studies should improve identification of race/ethnicity to include bi-racial participants.

Validity of Air Displacement Plethysmography

Air displacement plethysmography consists of a dual-chamber, sealed compartment with an oscillating diaphragm which enables the device to quantify body volume utilizing Poisson's law [36]. ADP assumes constant hydration, density of FM (0.9007 g/mL) and density of FFM (1.100 g/mL) which may be violated in racial/ethnic minority samples [30,138] . ADP is widely used in athletic facilities and laboratory studies including various races and ethnicities. To improve validity, population-specific equations have been created to estimate FM, FFM and %fat. However, few recent studies have evaluated racial/ethnic minorities utilizing race-specific equations.

Validity within Multi-Ethnic Samples

In a sample of 25 white and 39 black males, race did not affect the accuracy of ADP compared to a 4C estimate (White: r=0.59, SEE=5.3%; Black: r=0.76, SEE=4.7%) [22]. However, ADP demonstrated poor validity and underestimated %fat for both races compared to the criterion. In a smaller sample of white (n=39) and black (n=3) females, ADP %fat demonstrated good validity compared to a 4C model (r^2=0.92, SEE=2.68%), although the very small number of black females in the sample may limit the relevance of this finding to multi-ethnic populations [34]. Several investigations have evaluated the validity of ADP using DXA as the criterion. In a sample of overweight/obese females (White: n=17; Black: n=7), ADP FFM and %fat estimates were not significantly different than DXA measures (FFM: MD=0.98 ± 2.92 kg, r=0.90; %fat: MD=1.56 ± 3.75 %) [154]. An investigation of white (n=88.6%) and Asian/Asian Americans (n=11.4%) determined ADP %fat utilizing the Siri and Brozek equations were significantly different compared to DXA; however differences varied based on BMI category (Underweight: MD=7.3 %, Normal: MD=2.4 %; Overweight: MD=-1.48 %) [71]. In multi-ethnic populations, the validity of ADP is variable depending on the level of body fat of the population, the body density equation selected, and criterion method utilized. Future evaluation of ADP should investigate validity compared to a multi-compartment model criterion in samples including all racial/ethnic minorities across BMI categories.

Validity by Race/Ethnicity

In 37 Mexican males and females (≥ 60 yrs), ADP %fat was not significantly different than a 3C criterion (Siri et al. [123]) and had excellent validity (r^2=0.97, SEE=1.39%), however when evaluated by sex, males had significantly more variability in individual differences between methods (LOA=-4.4 – 2.5%) compared to females (LOA=-3.2 – 1.13%) [6]. Similarly, in 202 older Mexican adults (60-89 yrs), ADP FM estimates demonstrated very good validity compared to a 4C (r^2=0.93, SEE=2.3 kg) [5]. A study of 30 black males 19-45 yrs determined ADP had good validity for body density estimates compared to HW (r=0.91, SEE=0.00721 g/cc), and very good validity for %fat measures compared to DXA (r^2=0.86, SEE=2.84%) with ADP slightly overestimating %fat [141]. A large study of 445 Singaporean adults (91% Chinese ethnicity) found

ADP to significantly underestimate %fat compared to DXA (MD=3.9 %), however the methods were strongly correlated once adjusted for age, ethnicity, and BMI (r=0.93) [13]. An investigation of 50 Japanese males demonstrated similar %fat estimates between ADP and DXA at baseline (MD=-0.4 ± 2.8%; r^2=0.63, SEE=2.62%); additionally, following a diet or exercise intervention there was no MD between the change in (Δ) %fat (Δ% DXA: -3.9 ± 2.9 %; Δ% ADP: -3.9 ± 3.3 %) indicating ADP and DXA tracked body composition changes similarly following an intervention [113]. Very few studies have investigated race-specific validity of ADP in minority populations residing in the U.S. Additionally, to our knowledge no studies have investigated the validity of ADP in Native Americans or Pacific Islanders. Future studies should aim to evaluate race-specific validity of ADP compared to a multi-compartment criterion including both male and female minority adults residing in the U.S.

Validity of Bioelectrical Impedance Analysis

Single and multi-frequency bioelectrical impedance devices quantify total body water by measuring the resistance of body tissue as an electrical current passes through the body [36]. FFM can then be estimated by assuming a constant total body water (TBW)/FFM ratio of 0.732. Bioelectrical impedance analysis estimations of TBW have previously been validated against isotope dilution as the criterion [8,115]. However, BIA devices, notably those that employ a single frequency, are dependent on population characteristics such as age, race, sex and training status, which may vary greatly when assessing multi-ethnic populations. Multi-frequency BIS devices do not depend on population characteristics by utilizing Cole plot analysis of impedance (reactance and resistance) at multiple frequencies; BIS assumes specific coefficients for resistivity of tissue, body proportion and body density. Several investigations have evaluated the validity of bioelectrical impedance devices, primarily focused on the validity of regression equations

(Appendix A) created in large populations [i.e. Segal et al. [118] and Lukaski et al. [72]] for use in special populations including various races [15,129], elderly [41], children [46,124], overweight/obesity [106] and diseased states [35]. Initial studies validated BIA %fat and FFM measures utilizing HW as the criterion method, however more recent investigations have used DXA or a multi-compartment criterion. Bioelectrical impedance devices are commonly used as a field-based technique within the fitness industry and athletics as well as in laboratory and clinical settings.

Validity within Multi-Ethnic Samples

A large study in Native American (n=247), Hispanic (n=111), and white (n=244) adults (18-72 yrs) evaluated the validity of previously published BIA equations for estimates of FFM and reported SEE of 2.22 – 5.21 kg, TE of 2.28 – 7.23 kg, and r^2 of 0.73 – 0.89 compared to the HW criterion [128]. A recent investigation utilizing a 4C model criterion evaluated an 8-electrode, multi-frequency BIA device (Seca Medical) regression equation in a multi-ethnic U.S. population (n=130; Hispanic, Asian, black and white) and reported TE between 1.9-2.2 kg for FFM and TE of 1.3-1.7 kg for TBW [15]. A study in 100 children and young adults (8-21 yrs) residing in the West Indian region (Afro-Jamaican, Asian and European ancestry) observed BIA (RJL Systems) %fat estimations to be acceptable compared to HW (r^2=0.77, SEE=3.7%) using manufacturer equations [161]. More recent investigations have aimed to establish and validate regression equations in multi-ethnic (black, white, Hispanic, two or more races, Pacific Islander, Asian) samples of adolescents [46,124] and adults [127], and found including race as a predictor variable improved accuracy. However, a consensus on the most appropriate regression equation to minimize mean and individual error has not been established.

Validity by Race/Ethnicity

Several studies have investigated the validity of BIA in Asian populations including Chinese, Indonesian, Malay, Indian, Singaporean Chinese and Japanese participants. In 45 Indonesian adults, BIA demonstrated moderate to strong correlations (r=0.63-0.97) and large %fat MD (4.8 - 8.0 %) when compared to the Siri [123] 3C model [62]. Additionally, in 298 Asian adults (Singaporean Chinese, Malay, Indian), BIA demonstrated fair validity (r=0.87; SEE=4.5%) compared to a 4C criterion [26]. A study in 162 Indian adults males investigating the validity of %fat measured by leg-to-leg BIA (Beurer BF 60) and handheld BIA (Omron) found strong correlations (r=0.741 – 0.817) with DXA measures and no significant difference between the leg-to-leg BIA estimates (MD=0.72 %) and DXA, but a significant difference for handheld estimates (MD=4.44 %) [137]. A larger difference was observed in a study of 200 Indian adults between BIA and DXA %fat values depending on the race specific equation utilized (MD=5.4 - 8.3 %); both the Caucasian and Asian equations underestimated %fat [90]. Studies that have created BIA regression equations in Chinese and Southeast Asian populations have determined excellent validity for TBW (MD=0.0 ± 1.3 kg) when cross validated against an isotope dilution criterion method [48] and for lean body mass (MD=2.8 kg, r^2=0.97, TE=0.133) when validated against DXA [21]. Similar to multi-ethnic populations, incorporating race specific equations is important for valid estimates in Asian populations, but a consensus on the most accurate method may depend upon the country of origin and type of device used (i.e. handheld vs. leg-to-leg).

Previous investigations assessing the validity in individuals of African descent have used a variety of criterion methods, thus limiting translation of these findings. A previous study in black males (n=37) investigating BIA, using the Segal [118] equation, determined FFM was predicted accurately (r=0.97, SEE =2.1 kg) compared HW [143]. Another investigation of 20 black males utilizing BIA manufacturer equations (RJL Systems) determined BIA was not valid for %fat compared to HW (r=0.57, SEE=5.9%, TE=9.4%) [131]. More recently, a study including 250 North African adults cross validated (n=125) a newly created regression equation, and previously published equations compared to isotope dilution and reported variable error between equations for estimates of FFM (TE=2.46 – 4.10 kg, LOA: -8.71 – 7.03 kg) and TBW (TE=1.81 – 3.20 kg, LOA: -6.25 – 7.11 kg) [3]. In a similar investigation, five BIA equation estimates of %fat were cross validated with DXA estimates in a sample of 74 African American females and found poor validity for all equations (SEE=4.20 – 4.70 %, r^2=0.39 - 0.52) [70]. Bioelectrical impedance estimates in black participants have demonstrated poor validity, however, further investigations assessing validity compared to a multi-compartment criterion has not yet been explored.

In Hispanic populations, the validity of BIA estimates of body composition has not been thoroughly evaluated. A study investigating BIA estimates of FFM utilizing the Lukaski [72] equation determined a significant difference in Hispanic females (n=14, MD=-3.4 ± 2.6 kg, r=0.89), but not males (n=70, MD=0.54 ± 3.4 kg, r=0.89) compared to the DXA criterion; this could also be influenced by the small female sample size [35]. In 29 Hispanic females (n=22 were 100% Hispanic), several BIA equations were evaluated and demonstrated very good to excellent validity for FFM (SEE=1.4 – 2.0 kg; r^2=0.76 – 0.90) [129]. Previous investigations did not use race-specific equations for Hispanic participants, therefore a study in 155 males and females from Mexico (20-50 yrs) created (n=78) and cross-validated (n=77) a regression equation and found BIA FFM demonstrated good validity (r^2=0.92, MD=0.87 ± 2.84 kg) compared to ADP [74]. Similar to other ethnicities, current literature in Hispanic individuals suggests BIA race-specific equations should be validated against a multi-compartment model.

Validity of BIA in Native American participants has not recently been evaluated. Rising et al. [108] evaluated the validity of BIA FFM estimates using manufacturer software (SEE=6.89 kg, r=0.70) and a newly created equation in Native Americans (SEE=3.22 kg, r=0.92), and determined the race-specific equation improved validity from poor to acceptable compared to HW. A follow up study in 151 Native American females determined race-specific and general BIA equations overestimated FFM (TE=2.00 – 4.86 kg, SEE=1.69 – 2.8 kg, r=0.82 – 0.94) compared to a multi-compartment criterion [130]. Studies investigating the validity of BIA in individuals who identify as two or more races and Pacific Islander have not been studied separately in adult populations, and therefore future research should include these two understudied racial/ethnic categories.

Validity of Bioelectrical Impedance Spectroscopy

Few studies have investigated the validity of multi-frequency bioelectrical impedance (BIS) body composition measures in minority populations. A study evaluating black, white and Hispanic adults (n=150) reported that two tetrapolar BIS devices (Inbody 320 and Inbody 770) demonstrated significant mean differences in females (MD=2.99 %), but not males (MD=0.36 %), and poor validity compared to a 4C criterion (TE=5.0 – 5.5%, SEE=4.8 – 5.2 %, r=0.84 – 0.89) [45]. A study in African American college-age adults (n=143) showed BIS estimations were strongly correlated for FFM (r=0.911 – 0.918) and %fat (r=0.717 – 0.871) to ADP, however additional validity statistics were not reported [153]. Two studies evaluating multi-ethnic populations investigated the validity of BIS compared to isotope dilution, magnetic resonance imaging (MRI) [59] and DXA [84] in hemodialysis patients; thus, generalizability to healthy populations is limited. Future studies should evaluate the validity of BIS measures of TBW in minority populations compared to criterion dilution methods; the usage of BIS would eliminate the need for population-specific regression equations for TBW, like those used in BIA. However, as BIS body composition estimates still rely on assumed FFM properties (e.g., TBW:FFM ratio of 0.73), the validity of body composition estimates should also be examined even if BIS TBW estimates are deemed valid.

Summary and Conclusions

Although the minority population in the U.S. is increasing and is projected to become the majority by 2060 according to the U.S. Census Bureau, racial/ethnic minorities are still under-represented in body composition investigations [94,111]. Due to the relationship between body composition and cardiometabolic disease risk [12,86], it is vital to thoroughly investigate this component of health in minority populations and determine if current assessment methods are valid. Differences in body proportion, fat-free body density and hydration may have a larger effect on the validity of body composition devices in minority populations than previously assumed. Based on the review of literature, DXA is a valid method in a multi-ethnic sample, if individuals are Caucasian/White and African American/Black. However, there is insufficient evidence to recommend use in Hispanic/Latinx and Asian adults, Native American males, or African American/Black females. ADP is valid for Hispanic and African American/Black males when utilizing race-specific equations, however, results are inconclusive in other racial/ethnic groups and sexes. For BIA, body composition estimates may be valid in a multi-ethnic sample, but the literature demonstrates disparate results between races/ethnicities. BIA may provide valid results in Hispanic and Native American populations, as well as Asian populations utilizing race-specific equations. However, BIA is still not recommended for African American/Black individuals based on current data; although the lack of validation using a multi-compartment model criterion limits the certainty of this conclusion.

Before continued wide-spread implementation of each body composition device, there are several gaps in the existing body of research that should be addressed. For the DXA and ADP, there is a need for validity investigations that include larger and more racially diverse samples, specifically including Hispanic/Latinx and Asian adults, Native Americans, and African

American/Black females. For the DXA, in particular, technology has advanced significantly since the initial validity studies were conducted, and therefore conclusions are based on outdated models and software. For ADP, future validity investigations should utilize a multi-compartment model as the criterion as opposed to DXA, especially for Asian individuals. For bioelectrical impedance, additional studies validating BIS against a multi-compartment model are essential to ensure accurate results. Studies in more recent and improved BIA and BIS technologies should be conducted in Native American, Hispanic/Latinx and African American/Black individuals. Additional validity investigations may improve our ability to select the appropriate method to accurately assess body composition in each racial/ethnic population. This is essential for understanding disease risk in society as a whole and improving exercise and diet recommendations for disease prevention and management, as well as tracking changes from lifestyle interventions.

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Table 1. Subjective body composition assessment validity rating from Heyward and Wagner [49]*

Subjective Rating	%fat (SEE or TE)	FFM (TE or SEE)	
	Male and Female	Male	Female
Ideal	2.0	2.0-2.5	1.5-1.8
Excellent	2.5	2.5	1.8
Very Good	3.0	3.0	2.3
Good	3.5	3.5	2.8
Fairly Good	4.0	4.0	3.2
Fair	4.5	4.5	3.6
Poor	5.0	>4.5	>4.0

%fat = Body fat percentage (%); SEE = Standard error of the estimate; TE = Total error; FFM = Fat-free mass (kg); *adapted from Lohman 1992 [69].

Table 2. Validity of Dual-Energy X-Ray Absorptiometry in Multi-ethnic and Minority Samples of Healthy Adults

Study	Criterion	Outcome	N	Race/Ethnicity	Sex	Age (yrs)	MD (±SD)	TE	SEE	R^2	95% LOA
Wang et al [148]	6C	FM	23	Multi (12 White, 3 Black, 8 Puerto Rican)	74% M	44.5 ± 16.3	1.51 (1.1)	1.31	1.73	0.972	-4.0 - 3.4
Wang et al [146]	5C	%fat	27	Multi (14 White, 5 Black, 8 Puerto Rican)	78% M	43.8 ± 16.8	0.54 (2.4)	-	-	0.966	-
Prior et al [102]	4C	%fat	172	Multi (62 Black 110 White)	53% M	20.7 ± 2.6 (F) 21.2 ± 2.1 (M)	0.4 (2.9)	2.9	2.8	0.884	-5.3 - 6.1
Tylavsky et al. [136]	4C	FFM	58	Multi (6 Black, 52 White)	52% M	73.7 ± 2.2	2.8 *	-	1.6	0.98	-
	4C	FFM	13	Multi (2 Black, 11 White)	38% M	72.5 ± 1.2	2.6 $^{+}$* -2.7 $^{\#}$*	- -	- -	- -	-
Wagner & Heyward [140]	4C	%fat	30	Black	100% M	31.97 ± 7.71	-0.28	2.39	2.26	0.903	-
Collins et al [22]	4C	%fat	39	Black	100% M	23.8 ± 5.7	-0.2	-	-	-	-
Deurenberg-Yap et al. [25]	4C	%fat	291	Asian (108 Chinese, 78 Malay, 107 Indian)	51% M	36.2 ± 12.0 (F) 41.9 ± 12.9 (M)	2.1-2.5 * 3.2-4.2*	- -	- -	0.384[1] 0.314[1]	-
Hicks et al [51]	3C	%fat	147	Native American	100% F	34.5	0.3	3.27	3.28	0.785	± 6.4

MD = Mean difference; SD = Standard deviation; TE = Total error; SEE = Standard error of the estimate; LOA= Limits of agreement; 6C = 6-compartment model; FM = Fat mass; 5C = 5-compartment model; %fat = Body fat percentage; 4C = 4-compartment model; FFM = Fat-free mass; 3C = 3-compartment model; *Significant mean difference between criterion and DXA ($p<0.05$); $^{+}$Fan beam, $^{\#}$pencil beam; [1]partial correlation (corrected for age and ethnicity)

Table 3. Validity of Air Displacement Plethysmography in Multi-ethnic and Minority Samples of Healthy Adults

Study	Criterion	Outcome	N	Race/Ethnicity	Sex	Age (yrs)	MD (± SD)	TE	SEE	R^2	95% LOA
Fields et al. [34]	4C	%fat	42	Multi (39 White, 3 Black)	100% F	32.8 ± 11.0	-1.8 *	2.3	2.68	0.92	-
Lowry & Tomiyama [71]	DXA	%fat	64	Multi (57 White, 7 Asian)	78.3% M	55.0 ± 14.5	6.8 (4.4) (UW)* 2.4 (4.1) (NW)* -1.7 (3.3) (OW)*	-	-	-	-1.9 - 15.5 -5.6 - 10.3 -8.1 - 4.7
Wingfield et al. [154]	DXA	%fat FFM	24	Multi (17 White, 7 Black)	100% F	36.6 ± 12.0	1.6 (3.8) 0.98 (2.9)	- -	- -	0.44 0.81	-5.8 - 8.9 -6.7 - 4.7
Alemán-Mateo et al. [5]	4C	FM	202	Mexican	49.5% M	69.0 ± 6.4	-0.93 (2.3)	-	2.3	0.93	-5.6 - 3.8
Alemán-Mateo et al. [6]	3C	%fat	37	Mexican	59.5% M	69.3 ± 6.5	-0.99 (1.4)	-	1.39	0.97	-1.5 - 0.5
Collins et al. [23]	4C	%fat	39	Black	100% M	23.8 ± 5.7	-3.6 *		4.7	0.58	-
Wagner et al. [141]	DXA	%fat	30	Black	100% M	32.0 ± 7.7	-1.67 *	-	2.84	0.86	-
Bi et al. [13]	DXA	%fat	445	Singaporean (91% Chinese)	41.3% M	37.5 ± 14.5	-3.9 *	-	-	0.86	-2.3 - 10.2
Sasai et al. [113]	DXA	%fat	50	Japanese	100% M	47.8 ± 8.6	0.25 (2.9)	-	2.62	0.63	-5.52 - 6.02

MD = Mean difference; SD = Standard deviation; TE = Total error; SEE = Standard error of the estimate; LOA= Limits of agreement; 4C = 4-compartment model; %fat = Body fat percentage; DXA= Dual-energy X-ray absorptiometry; UW = Underweight; NW = Normal weight; OW = Overweight; FFM = Fat-free mass; FM = Fat mass; 3C = 3-compartment model; *Significant mean difference between criterion and ADP (p<0.05)

Table 4. Validity of Bioelectrical Impedance in Multi-ethnic and Minority Samples of Healthy Adults

Bioelectrical Impedance Analysis											
Study	**Criterion**	**Outcome**	**N**	**Race/Ethnicity**	**Sex**	**Age (yrs)**	**MD (±SD)**	**TE**	**SEE**	**R^2**	**95% LOA**
Bosy-Westphal et al. [15]	4C	FFM	130	Multi (31 Hispanic, 32 White, 31 Black, 36 Asian)	50% M	40.7	H: 0.4 (1.8)	1.9	-	-	-3.1-3.9
							W: 0.7 (2.1)	2.1			-3.4-4.8
							B: 1.5 (1.7)*	2.2			-1.8-4.8
							A: 0.7 (1.8)*	1.9			-2.8-4.2
Stolarczyk et al. [128]	HW	FFM$^+$	602	Multi (247 NA, 111 Hispanic, 244 White)	38.2% M	37.0 ± 13 (F)	0.14-3.17 (F)*	2.3-4.5	2.2-3.0	0.73-0.86	-
						33.5 ± 9.4 (M)	0.51-4.88 (M)*	3.6-7.2	3.6-5.2	0.76-0.89	-
Lopez et al. [70]	DXA	%fat	74	Black	100% F	47.6 ± 7.7	1.8	-	4.7	0.41	-
Wagner et al. [143]	HW	FFM	37	Black	100% M	30.8 ± 7.6	-3.3	2.7-6.0	2.1-3.9	0.79-0.83	-
Stout et al. [131]	HW	%fat	20	Black	100% M	21.0 ± 3.0	7.0*	9.4	5.9	0.32	-
Aglago et al. [3]	D2O	FFM	125	North African	22.4% M	18-64	-3.0- -0.04 (F)*	2.4-4.1	-	0.62-0.83[#]	
							-2.5-0.97 (M)*	2.6-3.9	-	0.64-0.76[#]	
Duerenberg et al. [26]	4C	%fat	298	Asian (140 Chinese, 72 Malay, 86 Indian)	49.5% M	36.2 ± 12.0 (F)	-0.7-1.5 (F)	-	4.5	0.76	
						41.9 ± 12.9 (M)	0.7-2.0 (M)*				
Kupper et al. [62]	3C	%fat	41	Indonesian	43.9% M	21.2 ± 2.9 (F)	3.5 (2.4) (F)*	-	-	0.56	-1.2-8.2
						28.1 ± 6.6 (M)	2.8 (4.3) (M)*				-5.6-11.2
Vasudevan et al. [137]	DXA	%fat	155	Asian (Indian)	47% M	45.1 ± 9.0	4.5[a] *	-	-	0.67[a]	-9.47-13.9
							0.7[b]			0.55[b]	-10.4-11.9
Nigam et al. [90]	DXA	%fat	200	Asian (Indian)	50% M	36.3 ± 7.5 (F)	-8.3 (3.9)[c]	-	3.5-4.1[c]	0.59-0.62[c]	-20.1-9.4
						37.1 ± 7.7 (M)	-5.4 (4.3)[d]	-	3.5-4.3[d]	0.54-0.63[d]	-20.6-11.4

Table 4 (Cont). Validity of Bioelectrical Impedance in Multi-ethnic and Minority Samples of Healthy Adults

Study	Criterion	Outcome	N	Race/Ethnicity	Sex	Age (yrs)	MD (±SD)	TE	SEE	R^2	95% LOA
Chen et al [21]	DXA	FM	209	Chinese	51.2% M	27.6 ± 3.0	0.7	-	-	0.94	-
		LBM					2.8	0.133		0.97	
Rising et al. [108]	HW	%fat	26	Native American	69.2% M	32 ± 10	0 (3)	-	3.22	0.85	-
							5 (7)		6.89	0.49	
Stolarczyk et al. [129]	3C	FFM	47	Native American	100% F	34.5 ± 9.9	0.7	2.57	2.38	0.8	± 4.9
Stolarczyk et al. [130]	4C	FFM	29	Hispanic	100% F	30.6 ± 5.5	-4.4 – 0.7	1.6-4.6	1.3-2.0	0.76-0.90	-
Macias et al. [74]	ADP	FFM	77	Mexican	47% M	34.0 ± 7.6	-0.9 (2.8)	-	-	0.92	-6.6 - 4.8
Forrester et al. [35]	DXA	FFM	84	Hispanic	83.3% M	34.4 ± 7.2 (F)	-3.4 (2.6)	-	-	0.79	-8.5 - 1.7
						36.9 ± 9.8 (M)	0.5 (3.4)			0.79	-6.1 - 7.2
Bioelectrical Impedance Spectroscopy											
Gibson et al. [45]	4C	%fat	146	Multi (50 White, 48 Black, 48 Hispanic)	50% M	33.1 ± 12.9 (F)	3.0 *	5.5	4.8	0.77	-
						30.6 ± 13.6 (M)	0.2 *	5.1	5.2	0.71	
Wi-Young et al. [153]	ADP	%fat	143	African American	44.8% M	20.0 ± 2.9 (F)	-0.2	-	-	0.52	-
						21.7 ± 3.0 (M)	-2.7			0.76	

MD = Mean difference; SD = Standard deviation; TE = Total error; SEE = Standard error of the estimate; LOA= Limits of agreement; 4C = 4-compartment model; FFM = Fat-free mass; H = Hispanic; W = Caucasian/White; B = African American/Black; A = Asian; HW = Hydrostatic weighing; NA = Native American; DXA= Dual-energy X-ray absorptiometry; %fat = Body fat percentage; D2O = Deuterium dilution; 3C = 3-compartment model; FM = Fat mass; LBM = Lean body mass; ADP = Air displacement plethysmography; [#]Concordance correlation (ρ_C); [a]Hand-held BIA; [b]Leg BIA; [d]Japanese-specific equation; [c]Caucasian-specific equation *Significant mean difference between criterion and bioelectrical impedance measure (p<0.05)

Appendix A.

Table 5. Equations for estimating body composition using bioelectrical impedance

Reference	Equation	Populations Evaluated
Lukaski et al. [72]	FFM (kg) = $0.756(Ht^2/R) + 0.107(Xc) + 0.110(BM) – 5.463$	Caucasian Adults, Hispanic Adults, African American Females
Segal et al. [118]	FFM (kg) = $0.00066360(Ht^2) – 0.02117(R) + 0.62854(BM) – 0.1238 (age) + 9.33285$	Caucasian, African American Males, Hispanic Males (%fat < 17%)
	FFM (kg) = $0.0008858(Ht^2) – 0.02999(R) + 0.42688(BM) – 0.07002(age) + 14.52435$	Caucasian, African American, Hispanic Males (%fat >25%)
	FFM (kg) = $0.00064602(Ht^2) – 0.01397(R) + 0.42087(BM) – 0.1238(age) + 10.43485$	Caucasian, African American, Hispanic, Native American Females (%fat < 25%)
	FFM (kg) = $0.00091186(Ht^2) – 0.01466(R) + 0.2999(BM) – 0.07012(age) + 9.37938$	Caucasian, African American, Hispanic, Native American Females (%fat > 35%)
Segal et al. [118]; Modified by Stolarczyk [128]	FFM (kg) = $[0.00066360(Ht^2) – 0.02117(R) + 0.62854(BM) – 0.1238(age) + 9.33285] + [0.0008858(Ht^2) – 0.02999(R) + 0.42688(BM) – 0.07002(age) + 14.52435] /2$	Caucasian, African American, Hispanic Males (%fat = 17 - 25%)
	FFM (kg) = $[0.00064602(Ht^2) – 0.01397(R) + 0.42087(BM) – 0.1238(age) + 10.43485] + 0.00091186(Ht^2) – 0.01466(R) + 0.2999(BM) – 0.07012(age) + 9.37938] /2$	Caucasian, African American, Hispanic, Native American Females (%fat =25-35%)
Stolarczyk et al. [130]	FFM (kg) = $0.001254(Ht^2) – 0.04904(R) + 0.1555(BM) + 0.1417(Xc) – 0.0833(age) + 20.05$	Native American Females
Rising et al. [108]	FFM (kg) = $13.74 + 0.34(Ht^2/R) + 0.33(BM) - 0.14(age) + 6.18(sex)$	Native American Females
Jakicic et al. [55]	BF% = $\{BM – [0.19(BM) + 0.20(Ht^2/R) + 11.57(Ht) + 2.55 (Eth. 1 = AA) + 2.68]\} /BM \times 100$	Caucasian and African American Overweight Females
Wang et al. [144]	BF% = $\{BM − [0.427(Ht^2/R) + 0.132(BM)+ 0.206(Ht) − 19.71]\} / BM \times 100$	Caucasian, African American, Asian Adults
Deurenberg et al. [29]	FFM = $- 0.34 (Ht^2/R) + 0.1534 (Ht) + 0.273 (BM) – 0.127 (age) + 4.56 (sex) – 12.44$	Asian Adults

FFM = Fat free mass; Ht = Height (cm) R= Resistance (ohms); Xc = Reactance (ohms); BM = Body mass (kg); %fat = Body fat percentage; Eth = Ethnicity; AA = African American

Appendix B

Figure 1A-H. Examples of two and three compartment body composition devices. A) BodPod®, COSMED USA, Inc., Concord, CA, USA; B) General Electric Lunar iDXA, Madison, WI, USA; C) Hologic Horizon A, Malborough, MA, USA; D) Single Frequency Bi-Polar, Hand-Hand Omron HBF-306C, Lake Forest, IL, USA; E) Single Frequency Bi-Polar, Leg-Leg Beurer BF22, Hallandale Beach, FL, USA; F) Multi-Frequency seca mBCA 514, Hamburg, Germany; G) Multi-Frequency SFB7 ImpediMed, Queensland, Australia; H) Multi-Frequency InBody 770; Biospace Co., Seoul, Korea

APPENDIX 2

Lifestyle and Diet Questionnaire Data

Table 1. Self-reported physical activity and dietary habits stratified by race and ethnicity. Presented as mean (standard deviation)

	Asian	**African American/Black**	**Caucasian/White**	**Hispanic**	**Multi-Racial**
Exercise (min/week)					
Moderate	118.9 (141.8)	98.9 (118.9)	189.5 (186.2)	156.0 (139.6)	156.7 (164.1)
Vigorous	61.9 (117.2)	82.2 (125.8)	42.5 (52.2)	53.6 (73.4)	49.8 (57.9)
Resistance	34.85 (7.31)	42.25 (10.12)	39.72 (8.55)	35.52 (8.72)	40.24 (8.70)
Diet					
Total Calories (kcal)	1796.4 (705.2)	2035.1 (544.7)	2133.6 (793.2)	2057.7 (628.1)	2025.2 (594.6)
Protein (g)	92.7 (9.3)	92.2 (34.0)	103.7 (44.2)	94.7 (45.1)	95.7 (27.9)
Carbohydrates (g)	194.2 (85.6)	217.2 (66.6)	248.7 (115.4)	242.8 (73.4)	228.3 (73.7)
Fat (g)	72.2 (31.9)	84.1 (34.6)	82.2 (28.5)	75.1 (24.9)	82.7 (32.6)
Sugar (g)	60.0 (37.6)	83.4 (47.2)	88.3 (54.0)	80.8 (30.8)	81.2 (56.2)

No significant differences between races/ethnicities ($p>0.05$)

Table 2. Education, marital status, alcohol consumption, and smoking history for total sample

	Frequency
Education	
No High School	1 (1%)
High School Degree	10 (9%)
Some College/Jr. College	22 (20%)
4-year College Degree	35 (32%)
Some Post-College	16 (15%)
Advanced Degree	26 (24%)
Alcohol Consumption	
Never	14 (13%)
<1 per month	22 (20%)
2-4x per month	54 (49%)
2-3x per week	15 (14%)
4+ per week	5 (5%)
Marital Status	
Single	75 (68%)
Married	18 (16%)
Living w/significant other	13 (12%)
Divorced	2 (2%)
Widowed	1 (1%)
Smoking	
Never	100 (91%)
Former	9 (8%)
Current	1 (1%)

Count (percentage of total sample); no significant differences between races/ethnicities (p>0.05)

Table 3. Frequency of stress level and sleep quality stratified by race/ethnicity

	Stress			Sleep	
	Low	**Moderate**	**High**	**Good Quality**	**Poor Quality**
Asian	12 (55%)	10 (45%)	0	7 (32%)	15 (68%)
African American/Black	10 (45%)	12 (55%)	0	10 (45%)	12 (55%)
Caucasian/White	16 (73%)	6 (27%)	0	14 (64%)	8 (36%)
Hispanic/Latinx	14 (64%)	6 (27%)	2 (9%)	9 (41%)	13 (59%)
Multi-Racial	10 (48%)	10 (48%)	1 (5%)	13 (62%)	8 (38%)

Count (percentage of sample within race/ethnicity); no significant differences between races/ethnicities (p>0.05)

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